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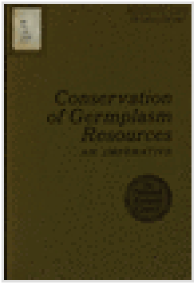

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AN IMPERATIVE

Committee on Germplasm Resources
Division of Biological Sciences
Assembly of Life Sciences
National Research Council

NATIONAL ACADEMY OF SCIENCES
Washington, D.C. 1978

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the Committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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PREFACE

In September 1976 the Assembly of Life Sciences, National Research Council, established a Committee on Germplasm Resources and assigned it these tasks:

- To study the status of germplasm resources
- To assess efforts to solve the problems associated with conservation of germplasm resources
- To prepare a report containing recommendations for making these efforts more effective.

The Committee has prepared its report for consideration by government officials, scientists (including those whose skills and experience are in areas not immediately related to the central issue of germplasm), and concerned citizens generally. The report is not intended to be encyclopedic, but underscores the fact that genetic diversity for many species is severely threatened and that, although considerable effort is already devoted to preservation of germplasm, much more emphasis is needed.

Soon after commencing its deliberations, the Committee sought help from a number of specialists in assessing the status of various types of organisms. Many responded graciously to these requests for information. We wish to express special appreciation to the following persons: Robert Bye, University of Colorado; Richard Donovick, Director, American Type Culture Collection; Norman R. Farnsworth, University of Illinois Medical Center; Tom Gilbert, National Park Service, U.S. Department of the Interior; Howard S. Irwin, President, New York Botanical Garden; Leon Jacobs, National Institutes of Health, U.S. Department of Health, Education, and Welfare; Robert Jenkins, Vice President for Science, The Nature Conservancy; H. E. Kennedy, Biological Abstracts; Stanley L. Krugman,

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Committee on Germplasm Resources

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1 INTRODUCTION

Germplasm resources may be defined as the total array of living species, subspecies, genetically defined stocks, genetic variants, and mutants whose continuing availability is important for society's present and future health and welfare. They may be regarded as the biological underpinning on which we live.

The earth is populated with millions of species of organisms that interact with one another to form complex ecosystems. Almost certainly there is considerable diversity within the gene pool of each of these species. Natural ecosystems and their component species have been evolving over millions of years without human influence and until comparatively recently have been unaffected by that influence. Not all the species in many natural ecosystems have been identified, nor do we understand many of the interactions in these systems; we find it difficult to assign them a precise quantitative value, anticipate the impact of human activities, modify that impact, and determine to what extent such perturbations are inevitable. Until recently the earth's natural habitats were so extensive that most people felt the preservation of whatever component species were of practical use to humans could be taken for granted.

With increasing industrial expansion and the rapid growth of human populations, incurring greatly increased needs for food, living space, and sources of energy, the world is losing many important natural habitat areas. We may be in great danger of losing species and variants that are essential to meet long-term human needs. There are likely to be, among these, species whose value we already know and others whose importance has not yet been recognized. Enlightened self-interest directs that we act immediately to prevent wholesale loss of resources that have evolved through millions of years of mutation and selection.

Environmental fluctuations and minor differences among similar ecosystems have led to the selection and maintenance of many gene differences among scattered populations of a given species of organism in nature. These differences constitute a rich source of variability that can be amplified through recombination and selection. The sum of these gene differences within a species constitutes the *gene pool* of that species. It is this natural variability that man has exploited in the past in developing domestic plants and animals from wild populations through selective breeding. It is extremely important that this naturally occurring richness of diversity within species be maintained. For this reason, efforts at preserving germplasm must look to the preservation of large populations of organisms, rather than merely a few breeding individuals.

Over a very long period, man has affected the genetic development of a significant, although relatively small, number of species through the selection of agricultural crops and domestic animals. We know that the gene pools of the "folk varieties" lying behind what are now major cereal crops contain many valuable genes that can be incorporated into modern genetically controlled stocks for such purposes as increasing resistance to specific pathogens and ensuring hardiness under difficult environmental conditions. Some of these genes from folk varieties, or land races, which have important positive effects in all environmental conditions, have already been selected for the development of genetically homogeneous high-production strains. Other genes, with effects needed only under special circumstances or in environments not encountered during the initial selection process, may not be incorporated into modern varieties. These "miracle" crops, which provide impressive yields under favorable conditions, have markedly increased cereal production in many parts of the world, thus helping to avert crises in the world food supply. This great accomplishment has its negative aspects, however: In some areas there is a clear danger that plantings of the new crops will completely replace plantings of indigenous folk varieties. Should this happen, important reservoirs of genes needed under less-favorable conditions will be irretrievably lost unless adequate provisions are made to guarantee their preservation.

Collections of varieites of economically important organisms serve as reservoirs of genes. Highly selected stocks of many economically important species have been produced and characterized. Their value resides both in the beneficial effects of the genes they carry and in the basic knowledge that results from the considerable human effort involved in producing and characterizing them. Many of these valuable stocks will be lost unless responsibility for maintaining them is assigned to competent curators and funds are provided to support the work.

Advances in biological research frequently depend on the availability of pertinent research material. In some areas of research, current choices of research organisms require species found only in the wild, and decreased availability of these species becomes an impediment to research. Research in many fields depends on supplies of organisms from special genetic collections. Certain convenient, well-characterized species are widely used in basic genetic research. Critical experiments using these species frequently require specific genetically defined stocks carrying known arrays of mutations or chromosome rearrangements. Specific enzyme deficiencies or alterations are required in some biochemical research. A considerable array of mutant experimental animals with counterparts of certain human diseases serve as valuable tools in biomedical research, and new models are still needed. One recurrent problem is that of providing for maintenance of genetic collections and mutant research colonies beyond the periods of the careers of the persons who established them.

Whatever the example chosen, it is clear that in each case the main issue has to do with preservation of the basic genetic material, DNA, which has the unique quality of carrying information, and the capacity of these sequences to replicate themselves exactly. As long as we have one copy of a particular gene, we have in principle the capacity to make more. Once *all* copies of a gene have been lost, resynthesizing its identical DNA sequence and integrating it with all the other pertinent genes to constitute the formula for a viable and useful organism is well nigh impossible. Co-adapted gene sequences, which are at least as important as single genes in this context, are even less amenable to manipulation and recreation with, say, radiation techniques.

Many U.S. agencies and organizations support programs designed to preserve genetic resources. Among the defects of the present situation is the widespread tendency for many important issues to "fall between the cracks" of existing commitments, such that no one takes full responsibility for facing up to them. Specific aspects of this situation are discussed later in this report.

Several federal laws and regulations relate to preservation of germplasm resources. The most familiar of these is the United States Endangered Species Act, enacted in 1973, which provides for maintenance of an official list of threatened and endangered species and bans any construction activities (e.g., building dams) that would damage their native habitats. Enforcement of this law has forestalled several ecologically undesirable projects. On the other hand, other laws, such as USDA importation rules, albeit effective in preventing infection of U.S. livestock with certain disease organisms, have had the undesirable effect of hindering highly relevant importations for scientific research. Overall, the body of the law that deals with genetic resources is diverse and without central focus.

Out of concern for the increasing numbers of species listed as endangered or threatened, we have considered how best to promote the continued existence of all truly endangered species. After weighing all available measures for preserving endangered species under controlled conditions, we are repeatedly forced to the conclusion that the only reliable method is in the natural habitat. Knowledge required to preserve all of these organisms under artificial conditions is not available and, if it were, this approach would be prohibitively expensive. Preservation in nature does not require that we understand it, only an awareness that it must not be destroyed. When preservation in nature is not possible, intelligent zoo or garden maintenance, with encouragement of breeding potentiality, offers a possible alternative.

We can hope in the future to be much more effective in preserving existing germplasm resources. Saving the rich diversity of genetic material that has been provided by natural mutation and evolution can be achieved and is worth whatever effort may be required. It is critically important that the people of the United States recognize the long-term dangers inherent in loss of specific genes and of genetic diversity, recognize that diversity in germplasm is an essential national resource, and treat it as such.

2 NATURAL ECOSYSTEMS AND BIOLOGICAL DIVERSITY

Significant human impact on natural ecosystems is a comparatively recent event. Modern agriculture is our greatest single ecosystem rearrangement, yet in the preagricultural situation of just 10,000 years ago we were hunter/gatherers playing a role in the ecosystem scarcely different from other animal species. Clearly all of the horticultural plants, domesticated animals, and useful strains of microorganisms upon which we now depend are the product of a very rapid and recent evolution under human selection from wild relatives found in natural ecosystems around the world. These ecosystems, undisturbed by agriculture, represent millions of years of evolutionary change and adjustment to changing life forms and environmental conditions. All forms of life have evolved within these natural systems; in turn, natural ecosystems have evolved to support the greatest possible diversity of living systems. A natural ecosystem with a diminished diversity of living systems is thus an impoverished system and any organism in a natural ecosystem with a contracting genetic diversity is a threatened organism. It is in the best interest of human society to see that the diversity of natural ecosystems does not appreciably diminish.

BASIS OF DIVERSITY

Natural ecosystems depend on an enormous number of environmental and biological interactions and interdependencies, and while many of them are highly fragile, yet they have survived for millions of years without human intervention. The welfare of each biological component within the community depends on the preservation of its genetic diversity, which variability in turn requires an adequate population size; the number of individuals needed to assure variability differs considerably from species to species.

To appreciate some of these complexities, consider the relationships of higher plants and animals to the many species of microorganisms with which they are associated. Certain microorganisms are essential to the well-being of a multicellular host; in many cases the host organism has evolved such dependency on its microbial symbionts that it cannot be regarded as an independent and self-sufficient entity. Consider, for example, the majestic Douglas firs of the Pacific Northwest and their dependence on certain fungi to form root mycorrhizae. Without soil fungi these trees simply will not grow. It is therefore essential that means adopted for the preservation of one plant also provide preservation for its symbiont. These complex interactions are usually best preserved in intact natural ecosystems.

At present, so few symbiotic relationships are understood in detail that it is simply not possible to decide, in most cases, which members of the microbial flora of plants and animals would need to be preserved separately to provide for healthy stocks of the host organisms. A substantive research effort would be required before such decisions could be made. Techniques for the isolation, cultivation, and preservation of many symbionts are not developed as yet. Most have not even been accurately described or identified.

Free-living microorganisms that inhabit soil and water are generally regarded as being ubiquitous. There does not appear to be much danger that any of these species of essential worldwide distribution face extinction. On the other hand the microbes that live in close association with plants and animals are in many cases highly adapted to this association. These highly evolved microbial symbionts can be expected to disappear when their host becomes extinct or is removed from its native habitat.

Another category of interrelationships is that between flowering plants and their pollinators. Adaptations are frequently so precise as to require the presence of both members of the partnership--plant and animal--for survival. Many of these situations are still unknown while hundreds have been discovered and intensively studied.

A commonly cited example of the importance of animal pollination--in this case by a wasp--is the initial failure of the fig to set fruit when introduced into the Great Valley of California in the last century. The trees grew well and all signs indicated that the crop would succeed, but the trees bore no fruit. What had occurred, of course, is that when the tree was brought from its native habitat along the Mediterranean the wild wasp pollinator was left behind. In the absence of its pollinator, the specialized flowers on the fig were nonfunctional. Only after research had uncovered the role of the wild wasp and it too was introduced, was successful cultivation of figs possible in California.

The survival of each species depends on the preservation of the intraspecific variability of the gene pool, plus its interrelationships with other organisms. No single organism possesses more than a fraction of the genetic variance of the species; thus populations are the smallest unit holding the genetic potential of a species. The maintenance of a species apart from its natural environment for significant periods of time is an uncertain means of preserving germplasm because of the expenses involved and because within a few generations the gene pool may become modified as a result of artificial and systematic selection pressures. The extreme examples are cultivated plants and domesticated animals, which have been so altered by selective breeding that they can no longer reproduce and compete in the ecosystem from which their wild ancestors originated. This loss of variability in crop plants has been further commented upon by Janzen (1973).

IMPACTS OF EXTINCTION

The loss of a species is an irreversible event terminating a process that has taken thousands or even millions of years of evolution (Prance and Elias, 1977). Replacement of this biological diversity through the evolutionary process will be extremely slow. The loss of a species will inevitably affect the ecosystem within which it existed. The ecological adjustments that are thus induced are difficult and often impossible to predict and may turn out to be deleterious to human welfare.

Man has had major impacts on natural ecosystems. These impacts include the destruction of many species of large mammals during the evolution of hunting and gathering cultures (Martin, 1966) and the early destruction of vegetation as a consequence of agriculture and overgrazing (Sears, 1935; Lowdermilk, 1953), which has been accelerated by technological developments that came in the wake of the industrial revolution and by the subsequent explosion of the human population, due in large measure to improved public health measures and to an agricultural abundance.

Certainly the greatest perturbation of natural ecosystems has been land clearing and the substitution of dense pure stands (monocultures) of cultivated plants and/or domesticated animals. In the past few decades, with rapid increases in yield achieved by employing hybrid crop varieties and new farming techniques, our dependence on future genetic improvements has increased rather than decreased. Over large areas of the globe the genetic uniformity of a few varieties and races is displacing the thousands of local gene combinations. This change has its most conspicuous implications in the less-developed parts of the world. The process represents a paradox in social and economic development in that the product of technology (breeding for yield and uniformity) displaces the resource upon which the technology is based (genetic diversity of locally adapted land races) (Wilkes, 1977).

Biological stability is in some measure achieved through genetic diversity. For example, in a wild population there exists in individual plants a fairly wide variation in their ability to withstand cold, drought, disease, insect damage, and other environmental variables. There are several ways to maintain stability in a predator-prey relationship, such as dispersing the prey at a low density so the predator will only occasionally encounter it; changing the prey from season to season; or ensuring the prey has great genetic diversity. In this last case, the predator will affect only a segment of the population. In most nonindustrial agricultures, there is normally considerable diversity between fields of different cultivators, as each maintains his own seed supplies. Suddenly, now, we realize that genetic uniformity is beginning to sweep around the world, as native varieties and breeds are dropped in favor of introduced seed or breeding stock. Quite literally the last vestige of the genetic heritage of a millennium in a particular valley can disappear in a single bowl of porridge. This genetic diversity was of no great value until it was the last, then suddenly it becomes immensely valuable.

Genes can be stored solely in living systems. Concern about their loss stems from the irreplaceable nature of genetic wealth. Once the systems are dead, the genes they possessed can no longer be retrieved. Wild plants can, of course, also be pushed to extinction by human activities, but the process is usually slower because it requires complete destruction. Indeed, although the American prairie, even after a century of land clearing and farming, is but a shadow of what it once was, much of the flora still exists. This is not the case with the native varieties and land races of cultivated plants, domesticated animals, and fragile habitats under human usage. Besides undergoing the slow process of genetic erosion, these biological units have been known to disappear in a single year. For this the term "genetic wipeout" has been used (Harlan, 1975).

Examples of recent man-made perturbations of natural ecosystems include the destruction of tropical rain forest by land clearing for agriculture; the replacement of natural forest by tree monocultures; pollution of water and air by industrial waste material; the obliteration of wild areas by urban sprawl; the modification of water courses (for power, irrigation, and flood control); the drainage of wetlands; the use of broad-spectrum pesticides and heavy applications of fertilizers; overharvesting of natural populations (e.g., whales, anchovies, tigers, passenger pigeons, and black walnut trees); and the deliberate or inadvertent introduction of species to new areas (e.g., chestnut blight and Dutch elm disease in North America or various species of mammals and cacti to Australia (Ricklefs, 1973).

Despite these many impacts of the human population, some relatively undisturbed ecosystems remain intact, even though in possibly modified and/or depleted form. They serve as self-sustaining reservoirs for a vast number of species, each with its natural gene pool. Thus it seems obvious that an adequate number of these ecosystems must be identified and appropriately protected if the world's germplasm resources are to be preserved.

The irreversible effects of extinction are not fully understood and may prove to be deleterious. The preservation of species in natural areas may keep open the options of ecosystem restoration at a later time.

It may not be possible to save several examples of every habitat, or the natural home of every species. Making decisions as to which habitats or which species are dispensable is extraordinarily difficult, if not impossible. Who would have guessed the value of a certain strain of *Penicillium* on a rotted melon in the Peoria, Illinois, vegetable market before the discovery of antibiotics; of *Rauwolfia* before the discovery of the action of reserpine (a tranquilizing drug); of the giant squid before the discovery of giant neurons and their use in neurophysiological research; and of certain species of insects and fungi as biological pest control agents before they were found to be useful. In the absence of specific knowledge, the best defense is the one exploited by natural ecosystems: genetic diversity.

3 PRESERVATION OF NATURAL ECOSYSTEMS

A report by the Nature Conservancy (1975) for the U.S. Department of the Interior addresses the issue of preservation of natural diversity in considerable detail. It underlines the desirability of setting aside natural reserves now, before diversity is further reduced by unwise land and water use. It recognizes that when its natural habitat is not preserved, a given species is more likely to become extinct. It advocates that a coordinated system of ecological reserves be established within the United States. We would add, also, that long-term monitoring of such reserves would facilitate distinguishing among cyclic, stochastic, and man-made changes in the system and thereby enhance capability for making predictions with respect to natural ecosystems.

The conservation of wild habitats is by no means the only aspect of ecosystem preservation; one must also consider artificially managed habitats, with their subclimax environments, many of which support a highly desirable and diverse flora and fauna. For such habitats there must be a clearly defined goal as to the desired state of the ecosystem and an intimate understanding of the way in which the elements of the system relate to one another, so that management can be directed toward maintenance of the system.

In estimating the cost of establishing and maintaining natural and artificially managed ecosystems, it must be recognized that such a program will provide society with much of value beyond the one addressed here: the preservation of biological diversity. Few attempts to estimate these values have been made, but an appropriate example is a study of the Alcovy River in Georgia (Wharton, 1970), which showed that the bottomland swamp forest was substantially more valuable in an undisturbed state than if if it were to be channelized and drained for agriculture. These assets included flood control, water purification, timber production, wildlife and fish production, and recreational and educational uses. Natural ecosystems serve vital functions in the maintenance of environmental quality. Protection of watersheds, recharge of the water table, and improvement of air quality are examples. Some of these areas are subjected to varying degrees of human use, ranging from more passive types of recreation (hiking, camping, canoeing, mountain climbing, and photography) through more active forms that have greater impact on the biota (hunting, fishing, and traversing by various types of motorized vehicles), to such exploitative activities as grazing and timber harvest. As for exploitation, management of ecosystems often conveys distinct advantages over "wilderness" natural ecosystems. Seed collecting for use in forest tree breeding programs stands as an example.

It is clear that any attempt to preserve biological diversity must confront the many conflicts of interest that arise. Common examples are: competition for winter range between elk and livestock; pressure for predator control programs as against their impact on wolves and mountain lions; hunting pressures and tourist safety programs in relation to the grizzly bear; drainage of wetlands areas for farming at key points on migratory flyways and on breeding grounds of aquatic species; and impact of massive use of pesticides on such nontarget organisms as bees, birds, and fish.

An essential feature of a successful program for the preservation of ecosystems and endangered species must therefore be the development of a cohesive plan for each reserve. The plan must include the enunciation of objectives and formulation of mangement policies to ensure that the objectives are achieved.

TERRESTRIAL AND FRESHWATER ECOSYSTEMS

Terrestrial ecosystems in the United States have been repeatedly classified and inventoried. An early attempt was published in 1926 (Shelford, 1926) by the Committee for the Preservation of Natural Conditions created by the Ecological Society of America. The magnitude and complexity of the problem are enormous, particularly when one is concerned with the presence of the rare components. Küchler (1964) has developed a vegetation map for the entire United States that divides ecosystems into major types. At the state level there are many natural-area inventories, information about which is readily available (Nature Conservancy, 1975).

Freshwater ecosystems--springs, streams, lakes, rivers, and their associated wetlands--are threatened in many ways. The threats take such forms as: pressures for use of the water for domestic, agricultural, and industrial purposes, which are particularly acute in the arid regions; manipulation of water levels for flood control and reservoir impoundment; dredging for navigation; drainage for agriculture; chemical and thermal pollution; solid-waste disposal; filling for real-estate development; and mission-oriented management for special interests (e.g., sport fishing). Goodwin and Niering (1975) have summarized the major human impacts on inland wetlands and have discussed their classification.

Populations in these aquatic ecosystems are frequently isolated because they cannot disperse widely, and a high degree of endemism therefore develops, especially in arid and semiarid regions. For example, endemic species of desert pupfish are found only in certain springs along the bed of the Amargosa River in Nevada (Miller, 1967).

The biota of aquatic habitats is not as well known as that of most terrestrial ecosystems. Hence, the dimensions of past and potential species extinction are less clear. Furthermore, the classification of aquatic habitats is not as well developed, although the U.S. Fish and Wildlife Service has devised a system for categorizing wetlands (Martin *et al.*, 1953; Shaw and Fredine, 1956). Preservation of freshwater organisms is especially difficult because information about them is inadequate and because their habitats are so often exploited to fulfill human economic and recreational requirements.

Any attempt to preserve freshwater organisms must take into account watersheds within the natural-areas system, as well as efforts directed to the restoration of water quality in our lakes and streams.

The Nature Conservancy promotes natural heritage programs on a state-by-state basis and guides their development. These programs are based on detailed inventories of the biota and emphasize rare species and plant associations. Further, the Conservancy has developed open-ended computer programs for compiling information that will permit the eventual development of a national data bank (Jenkins, 1977; Moyseenko *et al.*, 1977). The computer work has thus far been funded by modest grants from the National Science Foundation and private sources. Data gathering has been funded from state heritage program budgets.

MARINE ECOSYSTEMS

Species in the marine environment are by no means fully inventoried, although some communities, such as those occupying the rocky intertidal zone along the coasts of Europe and North America, are comparatively well documented. But the most diverse areas--the coral reefs and the deep sea--are very poorly known. In general, the marine environment has received much less attention than have the terrestrial and freshwater environments.

Diversity of species is, of course, the result of a continuing process of speciation and extinction. A general understanding of speciation has been arrived at for only a few groups of animals. It seems likely, however, that there are significant differences between groups of animals in the way in which speciation occurs, and great differences between environmental regimes in the rate at which species are formed and become extinct. It is generally believed that species diversity confers stability on ecosystems; recent theoretical analysis (May 1973) suggests that precisely the opposite may at times be true. It may well be that diversity does not always confer stability on a system, that the most diverse ecosystems are the most vulnerable to disturbance. In other words, such highly diverse ecosystems as the tropical coral reef and the deep-sea benthos may rely for their existence on environmental stability. Thus the very systems that are the least described and whose dynamics are least understood may be the most fragile of all.

Estimates of genetic variability indicate that species of marine invertebrates are in general highly polymorphic (Selander, 1976). Current evidence suggests, however, that variability is low in certain large marine crustaceans (lobsters and crabs) (Tracey and Nelson, 1975) and some estuarine species (Gooch, 1975).

One method for deriving comparative estimates of genetic variability is by electrophoretic survey of soluble products of structural genes. It is not known what proportion of the genome codes these proteins or what proportion codes the regulatory genes responsible for gene activation and expression. There are many instances in which morphological differences between species are not closely coupled with evolutionary divergence. For example, inshore and offshore populations of the well-known species of diatom *Thalassiosira pseudonana* are very different genetically, although similar morphologically (Murphy and Guillard, 1976). Comparable observations have been made on summer and winter populations of another diatom, *Skeletonema costatum* (J. C. Gallagher, personal communication). *Capitella capitata,* a cosmopolitan polychaete worm used as a pollution indicator, is now thought to be a complex of six sibling species that are morphologically similar, show almost no genetic overlap, and have sharply different life history characteristics.

Much of the genetic diversity in marine species resides in latitudinally separated populations whose systematic interconnections are unknown. An improved understanding of the role of planktonic larvae in linking discontinuous populations would help in elucidating the factors controlling the enormous year-to-year variations in recruitment of young that are so apparent in many commercial species of fish and shellfish. It is not now possible to distinguish between long-term cycles in the marine environment and unidirectional trends stemming from increasing effects of human activity. This distinction can be made only by instituting long-term studies of selected marine communities.

Although the major geographical boundaries in the oceans are well defined, to determine the degree of geographical isolation between populations in the marine environment is much more difficult than for terrestrial or freshwater systems. In many cases the presence of a particular species or community in a given body of water is the most informative indication available as to the history and physical and chemical properties of that water mass. Land-based and marine ecosystems differ greatly in the quality and quantity of information available at various levels of classification and inventory. Other important differences have to do with procedures for establishing and managing natural areas. Thus far, as a consequence, very few marine preserves have been designated. These differences may be partly due to the relatively recent recognition accorded to the desirability of conserving and preserving marine resources. The degree of recency can be appreciated by recalling that the national forest system was established in 1905 and that the first federal natural-areas site was set aside in the Coronado National Forest in 1927 (AIBS, 1974). By contrast, it was not until 1945 that President Truman proclaimed the natural resources of the continental shelf to be under the jurisdiction of the United States, and it was not until 1972 that Congress established an estuarine sanctuaries program as part of the Coastal Zone Management Act.

For several reasons, proposals for establishing an international system of reserves, with the primary objective of conserving genetic diversity, stop at the coastline, except for near-shore marine ecosystems in the Everglades National Park, Florida, and the Virgin Islands National Park. Plans of this nature were included in UNESCO's Man in the Biosphere (MAB) program and in the U.S.-U.S.S.R. Environmental Agreement (Franklin, 1977).

It may, indeed, be premature to expect comprehensive plans applicable to estuarine and marine environments. After all, not until 1974 did the Office of Biological Services, U.S. Fish and Wildlife Service, begin a major effort to revise and expand its classification of wetlands and aquatic habitats (including marine) with a view to producing a new inventory of wetlands. Odum *et al.* (1974) developed one classification of coastal ecological systems and subsystems, but a comprehensive assessment of estuarine and marine ecosystem preservation needs to be undertaken. The International Biological Program (IBP) was initially designed to obtain more information about the relation between biological productivity and human welfare, and in the United States included a section entitled Conservation of Ecosystems (IBP/CE), which in turn had an estuarine marine task force that developed a classification system for aquatic and related environments in the United States (Darnell *et al.*, 1974). Marine areas already designated for protection comprise primarily coastal lands. Adjacent and offshore waters were either not included or the administering body did not have regulatory authority over activities in those waters. The IBP terrestrial and freshwater task force did, however, manage to compile an inventory and assess the adequacy of representation of various ecosystem types (Darnell *et al.*, 1974). More recent attempts have been made to classify marine and estuarine habitats (Ross, 1974; Ray, 1975; and Kifer, 1975).

A number of baseline studies, now being conducted in the marine environment, should contribute to classification and inventory of marine ecosystems and lead to a deeper understanding of the dynamics of those systems. In the past, however, the expenditure of large sums of money on broad-scale sampling programs has not brought about such an understanding. Too often a piece of information crucially needed to make the whole set of data meaningful has been lacking, perhaps because there was no testable hypothesis guiding the sampling strategy. Funding policies should be such as to facilitate long-term studies by competent scientists and by institutions of established excellence, so that trends, periodicities, autocorrelations, and random fluctuations can be examined. Moreover, better communication should be established between federal agencies involved in environmental problems and ecologists engaged in fundamental research, such that the design of long-term data collection may be improved and existing data sets made more readily available.

It is clear that more effort needs to be devoted to gathering basic data on estuarine and marine ecosystems, especially at the classification and inventory level, and to long-term monitoring. Furthermore, legislation to preserve natural areas, and agency regulations and guidelines, should capitalize upon this information as it becomes available.

Long-term monitoring should be done on both unspoiled and perturbed areas so that predictions can be developed as to the probable effects of disturbance on different kinds of communities, ranging from the least to the most diverse. This approach should throw some light on the vexing question of the optimal size and number of areas to be designated for preservation (Diamond, 1976; Simberloff and Abele, 1976; Terborgh, 1976; Whitcomb *et al.*, 1976). Ecologists seems to agree that different species show different susceptibility to extinction. A strategy for preserving large, long-lived species at the top of the trophic ladder will obviously be different from one designed to protect relatively opportunistic species with high dispersal ability and short generation times. In general, the size of ecological preserves should be maximized, but a predictive theory for the fate of individual populations in, say, the marine environment requires much more information on larval dispersal, recruitment, and the frequency of local extinctions. It is likely that successful management will have more to do with biology of local populations than with efforts to encompass the total area.

THE PRESERVATION OF OBSCURE AND UNKNOWN ORGANISMS AND ENDANGERED OR THREATENED SPECIES

The preservation of habitats is not only the most convenient means of maintaining gene pools of many species of unknown value; it is the only means of maintaining the germplasm of unknown organisms. Because the larger and more conspicuous plants and animals, particularly in North America, have long since been catalogued, one may be inclined to think that the inventory of nature is nearly complete. But many of the more obscure organisms have yet to be systematically examined and can be classified only by general type. What few formal surveys have been conducted divide the paramecia, for example, into one of the dozen or more species complexes such as *Paramecium aurelia, P. multimicronucleatum,* or *P. caudatum*. Yet the likelihood is that each of these species complexes, if carefully examined, would be found to include several or many species that could be distinguished with the investment of appropriate effort (Sonneborn, 1975; Nanney and McCoy, 1976). This "under-classification" may be characteristic of the protists in general, of some groups of "uninteresting" plants, and of some of the less-conspicuous invertebrates. In many cases evidence for genetic discontinuity has never been sought. When diverse organisms are lumped together as a species, instead of being treated as a species complex, a particular component may be destroyed without ever being recognized or dignified with a Latin bionomial. The only possible strategy for the preservation of such species is the maintenance of a diversified system of natural habitats.

A special obstacle to preserving endangered species pertains with respect to organisms whose genetic economy is based on the exploitation of local habitats by genetic specialization (Sonneborn, 1957; Nyberg, 1973). Colonization and inbreeding produce many unique local populations, often largely or completely isolated from other related populations; hence they "speciate" freely into many local gene pools. Other organisms reflect the strategy of physiological responsiveness to adjust to environmental variables. When they outbreed, these organisms differ little in their genetic components over broad geographical areas; they rarely speciate, and their preservation is relatively easy.

The difficulty stems from the inflexibility of current legislation concerning endangered species. The loss of a "local" species of the *Paramecium aurelia* complex would probably be a far less significant event than the loss of a widely distributed species of the *P. bursaria* complex, but regulations do not now permit such distinctions. If one knew the propensity of certain kinds of organisms to speciate locally, one could probably discover a unique species in any habitat whatsoever chosen for preservation. Because relatively few of the local species have in fact been identified and named, the demonstration of a previously unknown species in an area perforce gives that area an aura of "importance." Although the tactic of using endangered species to block public works is often pragmatically feasible on a short-term basis, it is ultimately self-defeating.

The legislation must eventually be refined sufficiently to permit a more realistic assessment of the competing values involved. A realistic assessment must include an understanding of species diversity and species multiplicity in outbreeding and inbreeding species. A change in emphasis from protecting single endangered species to conservation of the total diversity in representative ecosystems would in part mitigate current weaknesses of the Endangered Species Act.

The Endangered Species Act has provided a strong legal means by which man's impact on the natural environment can be halted or reversed. In many cases where cogent arguments about postulated undesirable effects of environmental modification on whole ecosystems have failed to persuade, the Endangered Species Act has served as a means of last resort. Many examples could be cited where preservation of the critical habitat of a single nonresource species has halted multimillion-dollar projects of long standing. For example, early in 1977 the Tennessee Valley Authority was enjoined from further construction of the almost completed Tellico Dam on the gounds that completion of the project would destroy the habitat of the snail darter (*Percina tanasi*), a small fish found only in the Little Tennessee River. In delivering the opinion for the United States Court of Appeals for the Sixth Circuit, Judge Anthony J. Celebrezze and two other justices wrote: "Whether the dam is 50 percent or 90 percent completed is irrelevant in calculating the social and scientific cost attributable to the disappearance of a unique form of life. Courts are ill-equipped to calculate how many dollars must be invested before the value of a dam exceeds that of the endangered species. Our responsibility under the Endangered Species Act is merely to preserve the status quo where endangered species are threatened, thereby guaranteeing the legislative or executive branches sufficient opportunity to grapple with the alternatives." It could be added that it is not just the courts that have failed to develop the means adequately to weigh the value of nonresource species; the scientific community is similarly lacking in criteria.

To distinguish between threatened and endangered species, it is necessary to know the size of the population, to assess the change in size with time, and to have an understanding of the life cycle, population dynamics, ecological relationships, and habitat requirements of each species within the ecosystem. Habitat requirements for migratory birds along the length of their flyway pose special international problems. The status of a species can change swiftly as a result of human actions. Thus, if an exotic species is introduced or allowed to escape, the species may have drastic effects on the native biota; or a species may be overexploited; or an insecticide may be concentrated in predators at the top of the food chain. For example, very efficient harvesting of Antarctic krill might disrupt the ecology of that Antarctic ecosystem and endanger less common components of the system dependent on the krill as food (Shapley, 1977).

The total number of endangered plant species is unknown. Because of the relatively small number of specialists, it is not now possible to determine whether a given organism is really endangered as a species or only as regards one ecotype (e.g., Monterey pine). The conservation of small shrubs and herbaceous species relies more on guesswork than genuine understanding of their biology. Data are needed not only on their ranges, but also on their "requirements." Weber and Johnston (1976) are among those who have compiled lists of rare and endangered plant species, but the distinction between a species of plant and a variety has brought about discrepancies between various listings.

An examination of the list of endangered and threatened wildlife and plant species in the *Federal Register* (Vol. 41, No. 208, 1976) indicates that certain animal groups are well represented and that others are wholly absent. The gaps are particularly conspicuous when one looks for estuarine and marine species. Apart from a few species of large, long-lived animals (e.g., whales, manatees, crocodiles, and the short-nose sturgeon), species from the marine environment are almost entirely missing, and marine invertebrates are not represented at all. This is due in large part to comparative ignorance of the status of marine species and in some measure, no doubt, to the difficulty of finding advocates for small, insignificant-looking organisms.

Lists of endangered species and their habitats must be founded on an adequate data base. There should be a worldwide inventory of all types of organisms, of which a comprehensive national inventory for the United States would be an important part. Conflict with public works projects might well be avoided in the future if the habitats of endangered species had been thoroughly catalogued.

Were such a change in emphasis to occur, there would still be a number of endangered species, particularly certain large species of mammals and birds that will claim special rights to protection, either because they are of great aesthetic or economic importance or because they are a valuable research resource. The status of such species is considered by Zisweiler (1967), who has documented the accelerating rates of extinction that can be related directly or indirectly to the effects of human populations. The Red Data Books of the International Union for Conservation of Nature and Natural Resources (IUCN) provide an extensive analysis of mammals, birds, reptiles, amphibia, and fish in various degrees of endangerment. For such endangered species, guardianship in zoos or aquaria may be the only way of preventing their extinction; for other species, careful restoration of the natural habitat and a relaxation of the pressures previously leading to endangerment may be successful (e.g., the Hawaiian nene goose) (Martin, 1975); for others (e.g., the whales), extinction can only be prevented by the implementation of international agreements.

COOPERATIVE EFFORTS TO EXPEDITE CONSERVATION OF ECOSYSTEMS IN THE UNITED STATES

Public funds must be devoted to projects involving publicly owned areas. There is a need for coordination between action addressed to these areas and a number of other types of land holdings.

Many conservation organizations and institutions of higher learning own land characterized by managed or natural ecosystems. Some of these areas are being used for conservation programs; others survive through benign neglect. If all such areas are taken into consideration, there is very likely a larger capability for conservation than is generally recognized. Small-scale conservation efforts need to be publicized and help given to institutions, where needed, to preserve some segment of the ecosystem. Organizations involved in this kind of activity need to be made aware of the importance of their work and given an opportunity to participate in a network that would help to conserve habitats throughout the country.

In many cases the ecosystem harboring a given species may already be destroyed or unsafe. Banking in zoos or specific reserves has been instituted for some such species (e.g., Przewalski's horse and Arabian oryx) with the hope that they may ultimately be restored to their natural habitat. Rigorous restoration of the habitat and freedom from former pressures must be guaranteed before such repatriation is attempted. A comparable situation exists for many plant species, suggesting that botanical gardens and arboreta can play a role in preserving plant taxa that might otherwise disappear.

The effects of the loss of species and habitats are not fully known, and an argument for preserving diversity is often our ignorance of what would ensue if we neglect to do so. It would be unrealistic to expect to be able to preserve every living organism, no matter the cost, and a system of evaluation to facilitate decisions as to what should be actively preserved is urgently required. Further knowledge of species interactions would help to predict the consequence of extinction of one organism in an ecosystem. Until such knowledge has been developed it would seem prudent to make a major commitment to the identification and protection of as many ecosystems and endangered species as possible.

4 PRESERVATION OF ECONOMICALLY IMPORTANT PLANTS AND ANIMALS

THE VALUE OF COLLECTIONS

For centuries a wide variety of organisms have been collected and maintained as sources of food, clothing, shelter, transport, medicine, and research materials. Aside from the obvious convenience of having the organisms available when needed, collections make it possible to select for varieties that are better adapted to human needs than those found in nature. As a corollary to the selection process, many collections have been developed through the activities of investigators interested in the basic biology of the organisms, as such.

Many useful strains that have been developed through selection exist only in specialized collections, and such strains, which often represent a very large investment, are virtually irreplaceable if lost. However, the selection process can itself result in serious loss of germplasm. Selection for particular traits at the expense of others very often leads to a narrowing of genetic diversity in the species. The reduction in genotypes is particularly likely to occur as techniques for mass rearing are adopted and culture conditions become more uniform through artificial control. The discarding of all strains except those best adapted to the conditions of domestication becomes a serious threat to the maintenance of the organism if environmental conditions change, if susceptibility to predators changes, if parasites or pests develop, or if there is need to select for different sets of characteristics. Limiting the available types in a collection can be particularly serious if human or other encroachment on natural habitats has meanwhile depleted species diversity or has eliminated the species from natural communities.

ROLE OF PLANT GENETIC RESOURCES IN AGRICULTURE

In cultivated crops, germplasm resources are required to provide the genetic diversity needed to ensure future production. Plant genetic resources include wild species related to the cultivated crops, uncultivated forms of the cultivated species, folk varieties (or land races), obsolete and current cultivars, useful mutants, and stocks with improved combinations of genes developed as a consequence of research. This array of genetic diversity is essential to meet the constantly changing problems imposed by consumer needs, agricultural technology, environmental changes, pests, economic conditions, and other factors.

In the United States, responsibility for crop improvement through plant breeding is shared by the federal government, state governments, commercial firms, and foundations. Between 450 and 500 new cultivars are released each year. Most represent minor genetic advances and "fine-tuned" adjustments to changes in production, harvesting, processing, and marketing procedures.

Until about the beginning of this century, farmers commonly put aside plants from each crop for use in propagating subsequent crops. The saved plants were those judged to be superior. This practice has led to occasional hybridization (sometimes intentionally, sometimes by chance) and to many "folk" varieties. Substantial genetic variability existed within and among these cultivated varieties. Moreover, that part of a species not chosen for cultivation generally survived in nature, because its natural habitat had not been destroyed by the pressure of human population or by agricultural technology.

Early in this century the circumstances that existed for so long began to change; they continue to change, and rapidly. Professional plant breeding, generated by the rediscovery of Mendel's laws and the development of the chromosome theory of heredity, began 60 or 70 years ago. Application of these scientific principles led to modern crop varieties, which are selected for uniformity, yield, quality, and adaptation to specific environments. Selective breeding programs and the adoption of these superior stocks derived therefrom have brought about remarkable increases in agricultural productivity. Unfortunately, they have also led to the abandonment of many old folk varieties and land races, thus accelerating the erosion of plant genetic resources. In addition, the world population exploded from something over a billion to 4 billion people. The industrial revolution, coupled with population pressure, disturbed the natural habitats of many species, and as a consequence the genetic resources resident in wild and cultivated plants shrank. Important crops began to rest on a narrowed germplasm base, because farmers no longer saved their seed and, instead, obtained higher-yielding strains from a limited number of commercial suppliers.

NEED FOR A BROAD PLANT GERMPLASM BASE

Agricultural leaders in this country and abroad now recognize that genetic variability of crops is shrinking and that valuable plant genetic resources are thereby being lost. We cite now three examples of the dangers of a narrowed base; two deal with crises that were met in the past, the other with a current problem.

Grapes are among the economically more important fruit crops in the world. In the latter half of the nineteenth century the wine industry of France (and other European countries) was virtually destroyed by the ravages of an insect, *Phylloxera,* that attacked the roots of the vines. In Europe, vines were of but one species, *Vitis vinifera,* thus making all plantings on the continent equally susceptible to attack. By contrast, approximately 30 species are recognized in America, and it was known that the roots of some or all of these were resistant to *Phylloxera*. They did not however produce wine of quality equal to the European species. The numerous varieties of European grapes had been developed through centuries of cultivation and selective breeding from the gene pool of the one species, a process that could not be repeated rapidly with the American vines. The problem was solved, and the threatened demise of the industry avoided, by grafting the French vines onto selected American rootstocks, on which they still grow today. Similar combinations of highly selected vines grafted onto native American species stocks are also widely used in California. But these very native wild vines of North America are now being seriously reduced by the inroads of man.

A current situation that calls for diverse germplasm resources has to do with the reclamation of lands in the western United States that have been strip mined. In these semiarid regions the reclamation of strip-mine tailings is extremely difficult. Most of the native species best adapted to the natural conditions of these regions are not good colonizers of disturbed habitats. Some of the most promising species as candidates for introduction come from other regions and include weeds from other parts of the world. Yet reclamation with plants of this type carries appreciable risk because some of them may prove troublesome in the new environment. The solution to this complex environmental problem will require intensive research. It will require the testing of many species from many localities.

As a final example, consider the Southern corn leaf blight epidemic of 1970, which brought a new sense of urgency to the situation when losses at harvest reached 50 percent in some states and 15 percent nationally. This threat to a major crop created so much alarm that the National Research Council appointed a Committee on Genetic Vulnerability of Major Crops to examine the cause of the epidemic, the vulnerability of our crops to attack by pests and pathogens, and possible measures to hold losses to low levels and reduce the likelihood of epidemics. The report (Committee on Genetic Vulnerability of Major Crops, 1972) includes this statement:

> Two points are clear: (a) vulnerability stems from genetic uniformity; and (b) some American crops are on this basis highly vulnerable. This disturbing uniformity is not due to chance alone. The forces that produced it are powerful and they are varied. They pose a severe dilemma for the sciences that society holds responsible for its agriculture. How can society have the uniformity it demands without the hazards of epidemics to the crops that an expanding population must have?

In partial answer to the above question, the Secretary of Agriculture in 1975 established the National Plant Genetics Resources Board to advise the Secretary on national needs for the assembly, description, maintenance, and effective utilization of living resources in plant improvement programs. The Board is preparing an analysis of the status of crop germplasm resources in the United States.

In 1976 the Board established liaison with the International Board for Plant Genetic Resources, which was created in 1974 by the Consultative Group on International Agricultural Research. The mission of the International Board is to ensure the conservation of genetic variability in economic species of plants to be used by plant breeders and by research workers to promote improvement of cultivated plants and of agriculture itself. To this end the Board expects to develop collaboration among the members of a global network of institutions active in the exploration, collection, conservation, documentation, and use of plant genetic resources.

CATEGORIES FOR CROP GERMPLASM MAINTENANCE

Plant germplasm, in the broadest sense, includes all living plants capable of reproduction. Most species, particularly the uncultivated ones, have survived without any direct aid from human beings. This category (uncultivated species) includes wild relatives of the crop species. Habitat disturbance reduces the ability of the natural ecosystems to provide plant materials that may be needed in the future. Appropriate action is needed to preserve natural ecosystems.

A second category includes folk varieties, "dooryard" plants, and land races that merit some protection. They are in the care of small farmers, horticulturists, and gardeners in all parts of the world. No one has any inventory or fixed responsibility for them. They are part of the cultivated ecosystem.

A third category includes plants that have been assembled by scientists or amateur botanists. It is not unusual for individual scientists, employees of a research station, or employees of commercial seed companies to accumulate germplasm collections well beyond their immediate needs. Most are willing to share their stocks with others in an informal, uncoordinated system. Although this system is valuable, it is difficult to ascertain what stocks are available. It is vulnerable to losses as people retire and as administrators reevaluate priorities.

Most plant scientists feel that these three categories are not wholly adequate to meet crop germplasm needs. A fourth category, known as the National Plant Germplasm System, has evolved over the years.

THE NATIONAL PLANT GERMPLASM SYSTEM

The National Plant Germplasm System is a part of the USDA (ARS, 1977); the National Plant Germplasm Committee advises on its organization, administration, and operation. Although the system is fully operational and well established, the Committee has identified the following activities as meriting the earliest practicable attention.

Establishment of Repositories for Clonally Propagated Plants

Species that can be preserved by seeds are more easily managed than are those that must be preserved vegetatively. Some species (e.g., potatoes) can be preserved as seeds; however, the genotypes are so heterozygous that clonal propagation is also advisable for some stocks so that especially valuable genotypes will not be lost. Still other species must be clonally propagated because the plants do not reproduce by seeds. Compared with the seed crops, the conservation of clonally propagating ones is relatively uncoordinated and inadequately supported. The National Plant Germplasm Committee recognized that more attention must be given to clonally propagated plants. A start has been made by developing a national plan for maintenance of fruit and nut crops.

Establishment of a Tropical Facility

A facility for conducting research of special relevance to the tropics is needed. It should be in a latitude that provides the length of day required for flowering, in the field, of short-day or photoperiodic stocks. It should include a winter nursery where U.S. research workers can grow a second generation each year.

Funding Selected Curators

There are many individuals who maintain germplasm materials beyond their immediate needs and thus represent potential informal curators. Most are willing to share materials, but have had to support their curatorial activity out of research funds and, in an era of declining research budgets, this function has usually been the first to suffer. In the past few years, although funds have been provided to selected curators, most have not had any additional support. The National Plant Germplasm Committee found that many curators recognize their national responsibility for the germplasm in their care, which responsibility may have been channeled through regional or interregional projects or other cooperative agreements. But many other curators are holding germplasm because of personal interest or because they are working for an experiment station that undertakes to keep it as an adjunct to its research on some crop. They have in fact no formal responsibility to anyone and may dispose of the germplasm as they please. The Committee is attempting to determine who wants to serve as "formal" curators, i.e., persons and stations willing to assume responsibility for collection, maintenance, preliminary evaluation, recordkeeping or documentation, and distribution to qualified users.

The Committee will recommend specific funding for these curators.

Identification of Gaps in Major Collections

Support for foreign and domestic plant explorations comes from various sources, i.e., state, federal, and private funds. One account administered by the Agricultural Research Service, USDA, is used exclusively to support plant-collecting expeditions, about six per year. In 1976, for example, three foreign plant explorations and two domestic explorations were carried out. Germplasm of several species of cotton was collected in remote areas of Honduras, Nicaragua, and Mexico. Ecological data were also gathered on the boll weevil and other cotton insects. Primitive tomato varieties were collected in Panama, Costa Rica, Nicaragua, Honduras, and El Salvador. Citrus relatives for possible use as rootstocks and for breeding were collected in Australia and New Guinea. Some of the grapes native to the northeastern United States were collected and are being evaluated. Bitter gourd, a potential new oilseed crop for the arid Southwest, was collected from throughout the southern Rocky Mountains and Great Plains; the seeds will provide the basis for crop-development programs in several states. Other recent collections include grasses in Russia, legumes from Italy and Greece, potatoes and peanuts from South America, and sunflowers and pecans domestically.

Computerization of Information

Various elements of the National Plant Germplasm System have turned to computers as an aid in managing data on collections. For example, computers are being used by the USDA at the National Seed Storage Laboratory, Principal Plant Introduction Office, Western Regional Plant Introduction Station, and others. In 1976 a contract was let by USDA to assess various ways to develop a national system for storing and retrieving data, and suggestions sought from users and keepers of germplasm in an effort to design a system of optimum use to both groups.

Particularly useful would be expanded data on such characteristics of the stocks as quality, pest resistance, stress tolerance, growth habit, region of adaptation, and others.

Research on Techniques for Germplasm Maintenance

Long-term storage of germplasm is expensive. Research is needed to make storage more efficient, more secure, and less expensive. For example, research on the effect of temperature, moisture, atmosphere, and method of containment on longevity of seeds could lead to improvement in storage facilities and procedures. Too little is known of the cryobiology of seed, pollen, and tissue storage. Clonally propagated plants present a special challenge. Germplasm of fruit crops is maintained in orchards through whole-tree maintenance practices that require much land, labor, and money. If cells or tissues could be dependably stored and regenerated into whole plants, germplasm maintenance would be significantly less expensive. Much research will be required to make such procedures sufficiently secure to replace traditional methods.

INDIGENOUS SUBSISTENCE AGRICULTURE

Activities such as the National Plant Germplasm System are directed towards the agricultural needs of developed economies. The germplasm situation in countries relying on indigenous subsistence agriculture are often very different.

Subsistence agriculture depends on a reliable annual yield of crops, as much as possible irrespective of weather and soil conditions. This is in contrast with modern agriculture, which is geared to maximum yield of genetically uniform select cultivars that respond to definable water and nutrient levels. Native cultigens may be nutritionally superior and tolerate a greater diversity of environmental fluctuations. Analytical study of indigenous agricultural systems from ecological, genetic, and nutritional viewpoints would be valuable in assessing the need for conservation of this germplasm. If such systems appear nutritionally and ecologically sound for a given region, their survival should be encouraged, particularly where monoculture and the adoption of "green revolution" varieties is costly and may lead to meager returns because of uncertain soil and climatic conditions.

Any effort to incorporate indigenous subsistence agricultural systems into a genetic resources program must rest on the principle that genetic diversity is not only a function of the plants (past and present cultigens and progenitors) but also of the human element, which provides opportunity for expression of these variations and exerts selection forces to maintain genetic heterogeneity. The success of such a two-component process is a matter of survival for subsistence farmers. The imperative for assuring reliable yield on an annual basis in regions of poor and unpredictable growing conditions mandates the maintenance of very wide genetic variation in the food plants that are cultivated.

The impressive genetic diversity available in the food plants of the less-developed countries is now subject to the same pressures that have so drastically reduced diversity in the developed countries. Road-building, urban construction, and large-scale cultivation are reducing the land areas that contain the folk varieties of subsistence crops and their wild progenitors. In the case of some crops (e.g., wheat, *Triticum*), the introduction of the new cultivars produced in crop-breeding programs for large-scale production is eliminating many of the folk varieties or land races. It is well established that the genetic diversity of such food crops as white potatoes (*Solanum*), tomatoes (*Lycopersicon*), and sugarcane (*Saccharum*) has been severely reduced.

Only recently have efforts been made to conserve the genetic resources of the major crops characteristic of tropical agriculture. There are still no efforts to conserve those of lesser significance, such as the fruit and nut crops, of which there are a very large number. Vegetables, including many legumes, that are consumed by local peoples in the tropics are very poorly known. Even in the case of *Zea mays* (corn), for which germplasm has been collected extensively since 1943, we do not have as complete a collection as we should, and conditions for storage and rejuvenation are often far from satisfactory. This is due in part to failure to maintain valuable collections assembled within the past 35 years. They must now be reassembled, if that is still possible.

EXPORT CROPS OF THE LESSER-DEVELOPED COUNTRIES

A wide variety of export crops are produced on a large scale in the developing countries. These include tea, coffee, rubber, oil palm, cacao, teak, and other forest products. Major exploitation of some of these items is having drastic and deplorable impact on worldwide germplasm resources. The germplasm base of the crops thus exploited is being reduced through the planting of vast areas with single types, such that the enormous native diversity initially characteristic of these areas is being rapidly destroyed. The developed countries benefit from these crops in the short run, but their long-range interests are endangered by current practices. The developed countries should take some responsibility for curbing these inroads on the world's resources.

Rubber is an important export crop in many parts of the world but cannot be planted extensively in the Western Hemisphere because this is the native home of the plant (*Hevea brasiliensis*) and of a serious fungus pathogen of its leaves. Asian tropical areas are near peak production, and the introduction of the South American leaf disease could destroy this productivity. Because petroleum products are the starting material for synthetic rubber, it is probable that we shall become increasingly dependent on this crop. In any event, for some uses synthetic rubber is inferior to that from *Hevea*. It is therefore of special importance that a pool of diverse germplasm for this species be maintained as a resource for use in responding to future needs.

FOREST TREE GERMPLASM

Genetic diversity is fully as important for forestry breeding programs as it is for agricultural crops. If forestry operations are uncontrolled and ill-managed, and natural forest gene pools indiscriminately mixed, selected natural gene pools will be impoverished or even eliminated (Yeatman, 1972; Maini, 1973; Barber and Krugman, 1974). Forestry practices are becoming more intensive, and very few stands will remain untouched after a few more years. Native forests are often replaced by exotics or by nonlocal reforestation stocks. Thus we should try to maintain ancestral types in order to ensure that a broad pertinent genetic base is available for future selections.

The problems associated with maintaining a genetic base for forestry are not identical to those for general agriculture. Each tree encounters many environmental fluctuations during its long life span. Widespread species tend to be more genetically variable than are ones with restricted range, because of the greater diversity of environments encountered. Races of a species growing in different climatic regions may differ in their adaptation to environmental factors, but the limiting factors may differ from those governing cohabitation species (Callaham, 1970). Often there is little information about the most suitable seed source to use for a given location; hence, by default, local seed sources are employed.

To prevent loss of the original genetic base, strategies for maintaining a reliable and varied genetic reservoir for future improvement should be developed, and standards to gauge progress in genetic improvement and perpetuation of large and small populations for future mass seed production should be established (Yeatman, 1972; Maini *et al.*, 1975).

Protection of the gene pool of comparatively long-lived trees confronts fewer obstacles than does protection of annual crop plants. In addition, U.S. foresters, with a few exceptions, deal with wild populations of native germplasm. Difficulties that must be faced include preservation of diversity in areas of intensive management and the risk of losing germplasm from species with disjunct distributions, such as isolated stands and outliers. Some of the methods commonly used to maintain and protect forest resources include:

- Seeds, pollen, and tissue cultures may be maintained. Because not all material can be preserved, this approach necessitates a decision as to the future desirability of the varieties maintained.
- Selected stands may be set aside to preserve material considered to be essential. If this is not possible, plantations may be developed.
- Seed orchards and arboreta plantings are useful in maintaining genetic selections, especially those related to commercial forestry.
- Natural areas, national parks, and primitive and wilderness areas provide significant reservoirs of genetic diversity for forestry. They are, however, rarely established or managed for the express purpose of maintaining a broad genetic resource for forestry. Regulations as to the use of these areas often prohibit disturbance and

commercial activities, and therefore preclude mass seed collections for production forestry or related activities aimed at restoring damaged forest ecosystems.

Special gene pool centers for forest genetic reserves should be established. They should be representative of gene pools in areas where consumptive forestry is or will be practiced or where other pressures threaten the diversity. They should be large enough to contain the full range of biological and environmental diversity, to permit mass seed collections, and to minimize the hazard of contamination by foreign pollen.

Many current forest conservation programs include attempts to preserve the ecosystem in an unchanging state (e.g., by controlling forest fires). This may not always be desirable. Shade-intolerant species, important components of wood and fiber supplies, are often at a disadvantage in such undisturbed forest conditions. Management practices for genetic reserves should take into consideration whether it is advisable to aim for preserving an unchanging state.

DRUG PLANTS

Medicinal plants of importance fall into several categories:

- Those that yield pure chemical compounds of proven worth in the treatment of disease
- Those that are used in a crude or refined form and are therapeutically effective
- Those that yield chemical compounds of importance as starting materials for the semisynthetic production of useful drugs
- Those that yield extracts or compounds that in themselves have no appreciable medicinal value but may be necessary for the prepartion of drug formulations
- Those that yield chemical compounds of known structure, in themselves of no value as drugs, but useful as pharmacological tools in that they contribute to a better understanding of the mechanism of action of other drugs
- Those that have a widespread use as "herbal remedies" ("teas" and the like), in which case the medicinal value may well not have been established

In 1974 data were obtained from a National Prescription Audit (Farnsworth and Morris, 1976) as to the frequency of use of all drugs (synthetic and natural) dispensed from community pharmacies in the United States during the period 1959-1973. It turned out that prescriptions containing plant-derived drugs accounted for about 25 percent of all prescriptions and there is every indication that those containing plant-derived active constituents will remain at about that level for many years. In 1974 the retail value of prescriptions containing plant-derived active ingredients was about $3 billion. There is also abundant evidence that the "herbal tea" market, now at the multimillion-dollar level, will continue to expand rapidly.

If one groups the important drug plants--"important" in this context having reference to therapeutic effect--three categories emerge: (1) those of major importance as prescription drugs or as sources of prescription drugs (e.g., steroids, codeine, atropine, reserpine, quinine); (2) those of somewhat lesser importance that yield compounds used as prescription drugs (e.g., colchicine, papain, castor oil, cocaine); and (3) plants yielding materials used in pharmaceuticals in various ways (e.g., gum tragacanth, gum acacia, licorice, vanilla, peppermint oil, anise, strychnine).

It could well be argued that preservation of drug plant diversity is not only important as a reservoir for new and as yet unrecognized compounds, but that the relatively recent recognition of psychotherapeutic drugs lends a special urgency to this issue. In much the same vein, it appears that an intensified last look, so to speak, at folk medicines should be undertaken before these primitive societies wholly disappear, lest some effective remedies already identified in their cultures be needlessly overlooked. And it might be well to establish a screening program and inventory of herbarium specimens for medically active compounds, with special emphasis on ecotypes of areas that lie in the path of urbanization or agricultural development.

Not all materials of pharmaceutical value come from terrestrial flora, of course, although this is the traditional source. A number of highly active chemical compounds have been found to occur in marine species of plants and animals. They include antibacterial, antiviral, and tumor-inhibiting substances, some anticoagulants, and neurobiologically active materials. Many of these substances occur in tropical species, reflecting the fact that highly diverse ecosystems characteristically provide the greatest potential source of new compounds. Yet the biota of tropical coral reefs and the diverse fauna of the deep sea is almost totally unexplored in this regard.

LIVESTOCK GERMPLASM

The issue of livestock germplasm preservation has been thoroughly presented by Jewell, 1971; Bowman, 1974; Mason, 1974; Lauvergne, 1975; Rendel, 1975; and Bereskin, 1976. A variety of agricultural, scientific, and cultural justifications can be cited, but the principal ones bear on the potential for increasing the efficiency of food production.

Preservation of rare breeds assures a reservoir of genetic variability, unique genetic and physiological traits, and unknown genetic factors that provide genetic flexibility in meeting new demands and forming new breeds. Rare native breeds are often highly adaptable to special environments, including unfavorable environments where livestock production, in times of food surplus, has not previously been implemented. Rare breeds may be useful in crossbreeding to produce maximum hybrid vigor, to overcome lack of adaptability in highly productive breeds, or to meet changing product demands.

The question of preserving germplasm resources for livestock has received more attention outside of the United States than within. FAO reports of 1967, 1969, 1971, and 1973 considered many aspects of the problem and included some recommendations for improvement. Preservation is a larger challenge in older countries where native local breeds have been developed and selected, often over centuries, to meet special needs or to fit particular environments. Many of these breeds might be useful in increasing the efficiency of livestock production in the United States if ways could be found to import them without danger of introducing exotic diseases. Changing husbandry practices, such as the shift from species fed largely on grain to those fed on nongrain feedstuffs, increases the need for the importation of exotic germplasm.

The importation of livestock germplasm free of exotic diseases makes it possible to obtain unique genetic characteristics not present in domestic breeds, such as high fertility and milk production in sheep, high growth rate in goats, and high productivity in rabbits and other species. Breeds with high production efficiency but adapted to special environments in other parts of the world could be used in areas in the United States with similar environments. Gains from crossbreeding may well be enhanced by using exotic, highly productive breeds that differ genetically from domestic breeds. Preservation of germplasm in the country of origin, until the special characteristics are more completely measured and described, would be advantageous.

Mason (1974) has listed criteria that determine what breeds should be preserved, such as indigenous breeds, local productive breeds, genetically unique breeds, bizarre or beautiful breeds, and historically important breeds. Research needed for more adequate preservation of livestock germplasm includes cryopreservation of sperm, ova, and embryos and studies on superovulation, media, freezing, handling, storage, disease control, artificial insemination, and transplanting of embryos.

The preservation of livestock germplasm is in need of substantial support. The formation of a trust to preserve rare breeds in the United Kingdom has been described by Bowman (1974); it may be the most desirable solution to the problem, particularly if a permanent endowment with some involvement of public agencies can be established.

Breeds and strains in danger of being lost in the United States are considered more fully below.

Sheep

Three strains are in danger of being lost in the United States: Karakul, Old Type Navajo, and Southern Native. The Karakul is the only fat-tailed, fur breed of sheep in the United States. The Old Type Navajo was the only truly coarse-wool U.S. type that was adapted to very rigorous conditions; it appears to have been lost already. The Southern Native is unique in its adaptation to subtropical conditions and for its tolerance to internal parasites. It has been argued that the Southern Native might be saved if invasion by the coyote is halted.

Swine

At present there is nowhere a coordinated effort to preserve germplasm from breeds or strains of swine. Such an effort is needed, with initial emphasis on deciding which breeds or strains merit preservation. In addition, more research is needed to determine current production levels as a standard against which to measure yields in the future. An increase in the reproductive rates and a decrease in the generation time would reduce production costs.

Cattle

At present there seems to be no special concern about loss of strains. Still, research is needed to better evaluate strains and to preserve frozen germplasm.

Other Mammals

Preservation of the goat and rabbit probably should receive attention, although hard data are lacking as to need. It is assumed that preservation of the horse is assured by private industry, but this may not be true of work horses. Other farm animals, such as domestic dogs and cats, other pets, and fur animals have not been considered.

Poultry

The only currently successful method of maintaining germplasm stocks for birds is by maintaining breeding colonies. For chickens, turkeys, and a large number of other domesticated birds, this is accomplished by numerous fanciers throughout the country. Some commercial breeders retain up to 100 lines that they think might be useful in the future. Some universities maintain a few lines, usually not more than a half dozen or so, for their own work. The U.S. Department of the Interior is involved in propagating certain endangered species. There are now no germplasm stocks, and no plans for such stocks, in any federal agency.

Poultry producers badly need to use artificial insemination because, as a result of selection, some turkeys and meat-type chickens have such large breasts and are so heavy that they are unable to breed naturally. Natural mating results in extremely low fertility in turkeys.

Bees

There are probably 20,000 species of bees, worldwide. Most of these pollinate one or more species of flowering plant and are important as a source of honey.

In 1922 a law was passed in the United States that prohibited importation of adult bees except from Canada. It was designed to prevent the introduction of a mite that had been harmful to bees in Europe. Importation for research has been allowed occasionally.

There is wide genetic diversity among honey bees. Several years ago, a stock center was developed at Baton

Rouge in conjunction with the Bee Breeding Laboratory operated by Roberts and Macheson (ARS) and an effort made to accumulate representative stocks of bees. Inbred lines are maintained, and queens are sold from these for breeding or research purposes. Maintaining inbred lines is difficult because egg viability decreases rapidly with inbreeding.

There has been some success in maintaining and shipping semen, which should expand the gene pool.

PESTS AND PATHOGENS

Extensive research and development has been devoted to the eradication or control of numerous pests and pathogens. An understanding of the role that these species play in the balance of nature is of utmost importance.

At least some of the effort expended for developing pesticides and other control chemicals has been misdirected. Most pesticides adversely affect members of natural communities, including humans. It is becoming increasingly clear that more effective control of pests and pathogens must be sought through an understanding of their biology. This of course includes their life cycles, physiology, reproduction, population dynamics, and ecology.

The status of knowledge about pests and pathogens is still superficial, despite the volumes of research literature that have been produced. It appears that years of additional effort must be invested to achieve effective management and control of injurious species. A comprehensive collection of pests and pathogens would contribute significantly to research on biological control.

MARINE FISH

Fishes remain among the least known groups of vertebrates, despite their potential as a food source and the important roles they play in aquatic ecosystems (Mayr *et al.*, 1974). A large task lies ahead in completing the inventory of the fishes of the world. Many undescribed fishes are marine, but inadequately known continental areas, such as the Amazon Basin, doubtless have many unknown species.

Fisheries experts have documented many instances in which races or entire species of commercially important fishes have become extinct in a locality. In most of these instances, extinction can be traced to the activities of men. Usually, overfishing is not the sole cause; the situation is exacerbated by a combination of changes in the physical or biological environment that leads to species decline.

The complexity of the situation may be illustrated by the Atlantic salmon. One species is found throughout Europe and North America, but it is estimated to comprise at least 500 distinct stocks, many of which are found in a single tributary of a river system. The strong homing instinct in this species ensures that adults will return to their stream of origin, a behavior that contributes to the formation of distinct breeding stocks. Certain stocks have already been lost (e.g., in the Connecticut River), and difficulties have been encountered in restocking with fish adapted to other rivers. The situation is made worse by mass rearing of salmon in hatcheries, which in turn reduces the genetic base.

Genetic research on the Atlantic salmon and on other important fish species is of relatively recent origin. At the Atlantic Salmon Research Station (New Brunswick), directed by Dr. Richard L. Saunders and funded by Fisheries Marine Service, the Department of the Environment, Canada, a program has been established to examine the fine-scale variation in breeding structure and the range of genetic variation in the Atlantic salmon. The life history and migration patterns of stocks from different streams will be characterized. One aim is to develop a relatively opportunistic strain that can be used to colonize a variety of rivers from which unique stocks have already been eliminated.

Similar programs could be cited for other fish species with which scientists are working in an effort to recoup the loss of strains that were well adapted to given habitats. In all cases it appears that the species or stocks that can be maintained in hatchery breeding programs represent only a tiny fraction of the total genetic variability available in nature and that this variability can be preserved effectively only by preserving the natural habitats of as many species as possible. For certain species it may be found worthwhile to go to heroic lengths to preserve some portion of the germplasm in frozen banks of eggs or sperm, but this will require an intensification of research into criteria for cryopreservation of non-mammalian gametes.

MARINE INVERTEBRATES

The marine environment supplies not only fish but also lobsters, shrimp, crabs, clams, scallops, oysters, quahogs, and many other commercially important species. The list is long and undoubtedly could be extended by the exploitation of hitherto little-used species. The United States lags behind most other highly developed countries of the world in its use of aquacultural techniques for the improved management of these species, and the Committee on Aquaculture, Board on Agriculture and Renewable Resources, Commission on Natural Resources, National Research Council, is preparing a report that will list the reasons why this is so. A case study of the failure of many aquacultural projects in Florida (F. T. Mannheim, personal communication) indicates that the reasons are numerous, including certain legal barriers, the effects of vandalism, and environmental perturbations. Not the least, from the scientific point of view, is a lack of fundamental knowledge of the biology of the exploited species.

The most successful aquacultural projects have involved oysters and salmon, species whose biology has been comparatively well understood for more than 50 years. Nevertheless, culture of other marine species is being attempted, often without any fundamental understanding of the extremely high levels of genetic variability that characterize these species in their natural setting, and without an understanding of the consequences of inbreeding and selection under hatchery conditions.

It seems likely that few of the stocks of marine species that have been developed and maintained by individual workers would qualify for sustained long-term federal support (for example, from the National Science Foundation). Indeed, there are so few workers in marine genetics that most species have been studied by only a few investigators, some species by only one. Such stocks, no matter how faithfully they are maintained, do not receive sufficiently intensive use to justify federal funds. Yet, given the paucity of genetic information on marine species, it may be wise to develop a mechanism for ensuring some modest long-term support of genetic studies on marine species. Many of the food organisms used in aquacultural projects--for example, diatoms, *Artemia salina* (brine shrimp), and *Brachionus plicatilis* (rotifer)--have short generation times and would lend themselves well to genetic studies. Species of marine bivalves (e.g., oysters) have been maintained under hatchery conditions through many generations, but the genetic component in such studies has often been weak, or the stocks have had to be discarded for lack of sustained financial support.

5 PRESERVATION OF ECONOMICALLY IMPORTANT MICROORGANISMS

BASIC CONCERNS

When we considered the status of conservation of microbial germplasm, we were struck by two major concerns: lack of continuity of preservation and lack of ready availability of stocks. Both stem from organizational and managerial shortcomings in relation to existing collections of cultures. There is little reason to feel that current resources are grossly inadequate for present needs, but some gaps in coverage seem to exist, and others will certainly appear if existing resources are not conserved.

Lack of Continuity

Collections of great importance are at risk because there is inadequate organization at the national level. Such losses leave us in a vulnerable position in certain areas and are extremely wasteful.

Lack of organization incurs lack of continuity in the maintenance of collections. When no continuity is provided, collections suffer to an unnecessary extent from such things as changes in supervision, shifts in responsibilities and emphasis, death or retirement of curators, and shrinking research grants. Collections in government agencies are especially likely to deteriorate when breaks in continuity occur. These agencies assemble collections to meet a social need, or as preparation for tackling an acute problem (such as coping with a disease), or for use in conducting long-term research. When, on occasion, the collections are no longer needed for the purposes for which they were assembled, they may be discontinued. Universities seldom assume responsibility for maintaining collections assembled by their faculties, such that when an investigator dies or retires, there is too often no national repository to which his material can be sent, no agency responsible for arranging to have it continued, and no means to retrieve what is unduplicable material, whilst discarding the "usual."

In some areas, valuable resources are held in the laboratories of dedicated research workers who are attempting to respond to national or international needs, supporting collections on dwindling research funds at considerable sacrifice of their time and energy. If these resources were identified and their importance acknowledged, more realistic arrangments could be made. Clearly some organization at the national level is required.

Lack of Availability

Many existing collections are difficult to locate; often major collections are not generally available. This situation often leads to underutilization of resources and to costly duplication or hampering of activities elsewhere.

Many of our most valuable collections are used primarily as in-house resources. They are unpublicized, their holdings are not listed, and requests for access to them are often not granted. This is understandable, for they are not national, public collections, and staff and funds are not available for handling the distribution of stocks and providing the information that must accompany them if they are to be useful. The result is that these resources are unavailable to other institutions; either the holdings must be duplicated elsewhere, or research and development is hampered. The discarding of a collection in one laboratory is an intolerable waste if another laboratory is meanwhile busying itself assembling a similar collection.

In the past resources were ususally located by word of mouth or through notations appearing in published scientific papers. These procedures were satisfactory as long as the known organisms and the people working with them were few, but within the past 50 years these devices ceased to be adequate. No one can hope to keep track of more than a small fraction of the work published on microorganisms or to be aware of the location of more than a small fraction of the collections. Work with microorganisms is now conducted on such a scale, involves so many people, and affects society in so many ways that its management calls for improved organization. At present each large laboratory generally attempts to assemble its own collection and to be self-sufficient; the small laboratory is hard-put to find adequate resources to do this.

The question therefore is: How can we set up a more effective system to replace the traditional one, which broke down years ago?

One possibility is to create a single mammoth public collection of cultures or microorganisms for the United States. This was the solution envisaged 50 years ago when the American Type Culture Collection (ATCC) was founded. The organisms deemed worthy of preservation then numbered a few hundred specimens. We now know that microorganisms are extremely numerous, are extremely diverse (more so than any other group of organisms), and benefit society in many previously unsuspected ways. It is very likely that hundreds of thousands of specimens are worthy of preservation.

Is the approach exemplified by ATCC practical today? The most telling argument against it is that it probably would be impossible to assemble in one spot the curatorial expertise needed to handle the variety of organisms and the types of data that would have to be handled. It seems much more reasonable to build upon the existing scattered facilities as much as possible, seeking some consolidation and some overall organization to ensure long-term continuity and adequacy of coverage, availability, and quality.

PATHOGENS

The germplasm resources essential to the control of infectious diseases include large, comprehensive laboratory collections of pathogenic microorganisms. These organisms cannot always be obtained at will from nature and they are constantly needed in diagnosis of disease, for development of therapy and control measures, for selection or development of resistant host strains, for the study of epidemiology, and for the study of fundamental pathogenic mechanisms.

Because the task of preserving pathogenic microorganisms is worldwide, it can best be approached by organization at national and international levels. Such organization is needed to ensure adequate coverage and safety and to avoid excess duplication. The system must be sufficiently flexible to cope with changing social conditions, the movement of people, the introduction of new species, and the evolution of hosts and pathogens.

It is essential that the major collections be situated in laboratories having stable support and continuity of expert supervision. The need to preserve the agents responsible for past outbreaks of disease, including all significant variants encountered, makes for steady growth of these collections. Great care must be taken in deciding whether and when to reduce the holdings or to transfer their supervision, although constant culling is necessary, as in all collections.

The World Health Organization (WHO) has assumed responsibility for organization, at the international level, of germplasm resources essential for the control of infectious diseases of man. WHO has designated certain laboratories around the world as international and regional reference centers and has charged them with responsibility for maintaining reference collections of specific groups of microorganisms for the entire world (WHO, 1969).

About one-fifth of the centers described in 1969 were in the United States--in government laboratories, universities, and private institutions. To some extent the centers are supported by the institutions in which they are located and by grants from governmental and private agencies. Funds available from WHO are insufficient to support them.

In the United States a chaotic situation results from the fact that there are no national centers charged with responsibility for maintaining comprehensive culture collections of pathogens for use in the control and study of disease in man, plants, and animals. There are, indeed, large, comprehensive collections that serve these needs, but they are widely scattered; it is difficult to learn what their holdings are; and furthermore the holdings are subject to frequent and drastic changes. The collections have been built up in laboratories of the U.S. Public Health Service, the U.S. Department of the Army, the U.S. Department of Agriculture, and other government agencies; in university and other research laboratories; and in commercial laboratories.

MICROORGANISMS OTHER THAN PATHOGENS

Economically important, nonpathogenic microorganisms include:

- Symbionts of plants and animals of economic importance, which include some of the nitrogen-fixing species

- Agents used in food processing, as in the dairy and brewing industries
- Organisms that serve as direct food sources for man or domestic animals
- Organisms that may be used in controlling insects or other pests
- Organisms that are involved in food spoilage, or destruction of fabrics, fibers, forest products, and other materiel
- Organisms employed to process wastes (e.g., sewage purification, treatment of industrial plant effluents, conversion of wastes into useful products)
- Organisms used in producing chemicals (e.g., pharmaceutical products such as antibiotics and steroids, and industrial organic chemicals)
- Organisms used in monitoring the environment for the presence of harmful chemicals, such as mutagens
- Microorganisms used in applied and basic research

The germplasm resources on which man depends in dealing with nonpathogenic microorganisms consist primarily of collections of cultures in laboratories. These collections are a valuable investment. Many of the cultures are strains of microorganisms that have been selected and developed for specific economically useful properties, and therefore correspond to the crop plants and domestic animals among the higher organisms.

The loss of the working collections of microorganisms on which public health services, agriculture, and industry depend would lead to considerable disruption of these activities. Their replacement from nature would require years of effort and very large expenditures of funds. Thus it is essential to take such steps as will efficiently preserve what we have.

An evaluation of the major collections of cultures of microorganisms in the United States would be difficult, but it should be performed. Earlier efforts to compile lists of these resources failed to achieve complete coverage and did not assess the quality and value of the collections listed. It is impossible to describe the situation accurately on the basis of available information, but what is known suggests waste, inefficiency, considerable duplication in some areas, and perilously sparse coverage in other areas.

The ATCC, set up in 1925, has been noted above as the one widely recognized culture collection in the United States. It is a private, nonprofit organization supported largely by government agencies, by fees charged to recipients of stocks, and by fees charged to donors for maintenance or distribution of stocks. Although it contains a wide variety of useful microorganisms, it probably could not serve as the sole national resource for any group of microorganisms.

Some industrial collections are among the largest and most stable. A few have more extensive holdings, numerically, than the ATCC. An idea of the extent to which our society is dependent on industrial collections can be gained from a review of the fermentation industries producing pharmaceuticals and fine chemicals, worldwide (Perlman, 1977). This review lists 183 products, including 73 antibiotics, 18 tetracyclines, 27 enzymes, and 10 organic acids, plus solvents, vitamins, amino acids, and steroids that are produced by microbial fermentation. Some of these pharmaceuticals and chemicals are produced in quantities of 50,000-200,000 tons per year. Furthermore there is increasing interest in using microbial cells for protein in human and animal diets and in using microbial processes to generate chemicals now produced chemically from petroleum products.

The remaining microbial collections on which the nation depends are scattered in laboratories in government agencies, universities, and elsewhere. The questions of continuity and availability are similar to those afflicting collections of pathogens. These collections are difficult to locate, which in turn leads to underutilization and duplication of effort. Continuity often depends on the efforts of dedicated persons who accept responsibility for assembling and maintaining the collections and for obtaining financial support. Usually the collections are supported by funds that have not been specifically designated for that purpose. These collections, like those of pathogens, sometimes are discarded upon the retirement or death of the caretaker.

Certain factors contributing to this unsatisfactory situation are: (1) Lack of organization at the national level and consequent lack of support and clearly assigned agency responsibilities; (2) a general mistrust of centralization, which results in part from past instances of uncertain support and inadequate supervision; (3) failure to develop a system of data storage and retrieval adequate for the laboratories, activities, and data now existing; (4) an economic situation of some years' duration that provided research funds at a level such that extensive, but uncoordinated, culture collections could be built up and maintained on research grants not specifically designated for that purpose.

The prospect of greater dependence on microorganisms for food and chemicals lends urgency to the need to assess our resources and to provide for their use and preservation in a more efficient manner. The problem is exacerbated by trends in science education that have deemphasized broad biological training required for those reponsible for maintaining collections of broad coverage.

Some efforts to rectify the situation have been made. Internationally a start has been made in compiling a list of collections of cultures of microorganisms. A World Federation for Culture Collections (WFCC; formerly, Section on Culture Collections) has been formed within the International Association of Microbiological Societies of the United Nations Educational, Scientific, and Cultural Organization (UNESCO). WFCC sponsored a worldwide survey of culture collections with support from UNESCO, the World Health Organization, and the Commonwealth Scientific and Industrial Research Organization, Australia. A world directory was published as a result of this survey (Martin and Skerman, 1972). The 349 collections listed in the directory represent only a small fraction of the existing collections, but they are a beginning. Efforts are being made to establish an international information center to provide information on collections (see Skerman, p. 141, in Iizuka and Hasegawa, 1970). Coverage for the United States, however, is inadequate; many important collections are omitted and some trivial ones are included.

The difficulties encountered in conducting the survey indicate how great was the need (Martin and Quadling, 1970, p. 133). Even the microbiological societies of the various countries apparently were of little help in this respect. Those in charge of many collections did not respond to questionnaires, in part, perhaps from apprehension that to be listed in the directory would generate more requests for cultures than could be handled (Simmons, 1970).

UNESCO has also supported international conferences on culture collections. The first was held in Tokyo in 1968. The proceedings (Iizuka and Hasegawa, 1970) suggest that the United States is not meeting its needs for microbial culture collections as well as are a number of other countries (e.g., Japan and the United Kingdom).

The WFCC has placed considerable emphasis on the development of culture collections and the expertise necessary for managing them in developing countries.

6 COLLECTIONS FOR RESEARCH, TEACHING, AND PUBLIC EDUCATION

The status of collections of noneconomic significance varies widely, depending on the type of organisms involved and the use to which each collection is put. The size of a collection, the form in which it is maintained, its value in terms of the effort that went into its development, its future usefulness, and so on, are factors that must be considered. We must, however, recognize the value of collections in a general sense and the need to ensure their high quality and continuity.

Research collections of organisms used as models for exploring the fundamental properties of life are an irreplaceable resource because of the mutant strains that have been accumulated and the knowledge that has been developed in the course of their characterization. Because of these background data such strains can be used in the investigation of problems whose solutions would be of signal benefit.

Less widely used and more specialized collections, particularly those developed by a single investigator or fancier, are far less secure than collections of economically important organisms because much of the value is represented by the knowledge and skill accumulated by that one person. To recognize the value of such a collection is at times difficult; even when value is obvious, it is often difficult to find someone else able and willing to carry on the work.

Research collections, in addition to requiring specialized knowledge, raise a variety of problems as to quality control and maintenance. Some require elaborate conditions for culturing and preservation.

BOTANICAL GARDENS AND ARBORETA

The role of botanical gardens and arboreta as institutions of fundamental importance in the conservation of genetic resources is too frequently overlooked. Technically speaking, arboreta grow only woody shrubs and trees, whereas botanical gardens are not thus restricted; in practice, the differences are trivial.

Most who are familiar with the important botanical gardens and arboreta have been struck by the uniformity of their traditions and functions. In the Americas and Europe, if not elsewhere, the gardens are largely nineteenth-century institutions created to meet the demand for popular education in a period when world travel was restricted to the few. They parallel Victorian museums; in many respects museums and gardens evolved together.

Botanical gardens early became citadels of research on diversity and the cultivation and propagation of plants. They were not thought of as instruments of plant conservation, mainly because at the height of their expansion Victorian optimism extended over the whole field of world resources, which then seemed inexhaustible. Although rare plants were often cultivated, there was little concern for preservation of the stock, and records were hardly ever maintained. Unfortunately, most gardens continue to operate on this level; such conservation role as they fill is ususally secondary and is seldom well documented. An increasing number of botanists deplore this state of affairs, and their swelling ranks now include some who direct botanical gardens and determine their program priorities.

What in fact can botanical gardens and arboreta do to promote a better understanding of the endangered-species issue and actually to conserve genetic resources? By their very nature they are treasuries of botanical diversity, growing everything from algae to redwoods and displaying a wide array of native and exotic species and cultivars. It is not uncommon for them to maintain several thousand species in a score of natural or simulated habitats. Regardless of the quality and extent of documentation, the mere fact that such an aggregation of genotypes is consistently maintained gives it conservation value and argues for a more direct role in botanical conservation. Several gardens also own, or have custody of, significant tracts of natural vegetation that promote public understanding, serve as refuges for the genetic resources found in them, and make these resources available for research.

Historically, botanical gardens have often been very active in distributing plant materials. Most have an *Index Seminum* and have participated in international exchanges on a considerable scale.

Associated with the living collections of many botanical gardens are libraries devoted to botany and horticulture. Several institutions also maintain plant-specimen museums, or herbariums, which are collections of well-documented specimens representing a very diverse flora. Thus gardens and arboreta and their associated facilities are essential instruments in research in plant systematics, evolution, geography, and ecology. Such research is fundamental not only for understanding the forces that accelerate impoverishment of genetic resources, but also for establishing sound conservation practices.

Botanical gardens customarily offer educational programs to an increasingly interested lay public. Several, either as components of or in association with universities, provide professional and postgraduate training in botanical science and in various aspects of horticulture. Both activities provide a sound basis for developing a stronger conservation focus.

Public Exhibits

Gardens and arboreta should develop public exhibits dramatizing the need for botanical conservation. In this respect, the custodians of the plant world have much to do. Few are aware that one in 20 native plant species in North America is threatened with extinction. Displaying and interpreting rare plants could give enormous publicity to local, national, and international conservation organizations. By responding to such questions as why some plants are rare, what factors threaten their continued existence, and why botanical diversity is important, gardens could do much to help organizations involved with the preservation of natural resources. No other group is better able to espouse the cause of botanical diversity.

Research Emphasis

Botanical gardens and arboreta should broaden or shift their research programs to include studies on plant ontogeny, longevity, and reproductive effectiveness, focusing these investigations on rare plants. Information of this kind is essential to any attempt at salvaging plant species threatened with extinction by bringing them into cultivation. So little information of this nature is available that attempted salvage often fails. Simple, well-documented cultivation experiments, preferably in conjunction with a nature preserve, would do much to provide information on species of narrow ecological amplitude whose survival is dependent on cultivation in alien habitats.

Preservation of Endangered Species

Botanical gardens and arboreta should do a better job of safeguarding stocks of endangered plant species. Critical to this function is a satisfactory system of documentation for living collections. Wild species in cultivation, and all seeds and other propagating materials sent out on exchange to other gardens, should be of known and recorded origin. Propagation of rare and endangered species should be attempted not by transplanting (unless this is the only remaining option), but by judicious seed or cutting collection. American botanical gardens and arboreta are the most logical agencies to take on the responsibility of protecting the thousand-odd plant species proposed for threatened and endangered status in the United States. Special attention should be given to species actually facing extinction, to narrowly endemic species confined to a particular limited area, and to wild relatives of important cultivars. Some species may lend themselves to seed-bank conservation; others must be maintained in successive generations. Either approach should be undertaken with a view to eventual reintroduction to the wild, where possible.

Because some larger gardens have long-standing involvement with programs in the tropics, where both speciation generally and the difficulty of protecting rare and endangered species are greater (in part because so little is known about them), they might well emphasize the preservation of selected tropical groups. International coordination is obviously essential to success in such a complex endeavor, and several conferences addressing this topic have been held in recent years.

A number of questions confront botanical gardens and arboreta with respect to threatened and endangered species:

- How can arboreta and gardens serve as salvage centers for species otherwise certain to become extinct?
- How can the already slender and diminishing financial

resources available to the gardens and arboreta be directed to such a purpose?

- How can the salvaging of threatened and endangered species serve more than an archival function?
- How can arboreta and gardens assist other agencies in preparing for the reintroduction of salvaged species into the wild?
- How can arboreta and gardens function in enlarging the catalog of economic plants?

Botanical gardens face new challenges and problems that cannot be met in the old way. The new spirit requires integrated, coordinated efforts by the world community of botanical gardens and arboreta. That approach is given impetus by the increasingly prominent roles played by the United Nations (as in the Man and the Biosphere program) and by the International Union for the Conservation of Nature and Natural Resources (IUCN), the World Wildlife Fund, the Nature Conservancy, the National Audubon Society, the International Association of Botanic Gardens, and others.

ZOOS AND AQUARIA

An increasing number of wild species face extinction. The reasons for this threat are detailed by Zisweiler (1967) and Myers (1977), who provide cogent arguments why the loss of species is of serious concern.

Zoos and aquaria now safeguard some of these species; others can find a refuge if sufficiently coordinated efforts are made. But for many animal groups it is impossible thus to avoid their extinction. Extinction of whales and certain other marine life can be prevented only through international agreements. Some migrating species of birds, e.g., hummingbirds and the whooping crane, can be conserved only by preserving their very limited habitats. Ecological niches are so specific for some species, and their reproduction in captivity is so complex or poorly understood (e.g., bats and the platypus), that preservation of wild habitats is the only means of ensuring preservation. The national refuge system, the national park system, and various other schemes seek to meet the need, but in many cases the ecosystem harboring a species is already destroyed or unsafe.

Zoos and aquaria represent a substantial industry; the United States alone has 230 listed in the *International Zoo Yearbook* (Olney, 1976). Together, they hold many species that have become extinct in nature (best known are Pere David's deer, Arabian oryx, and Przewalski's horse) and others that are severely threatened.

Intensive efforts have accomplished reintroduction of certain species to their original habitat. The Hawaiian goose (*Branta sandivicensis*) is a good example (Martin, 1975). In 1700 there were about 25,000, in 1940 only 43. Today, as a result of captive breeding, there are over 3,000 in the wild and 200 in captivity.

Traditionally, zoos have replaced losses by purchase from dealers, but in the last decade serious efforts at conservation have been started, and reproduction of threatened species in captivity is of primary concern to modern zookeepers. The International Species Identification System (ISIS), a computerized program located at Minnesota State Zoo, gathers data on animals held in zoos and aquaria for breeding purposes. Over 18,000 are now recorded, from 111 participants. Two international congresses on breeding endangered species in captivity have been held (Martin, 1975; Olney, 1977).

The accelerating rate of extermination is correlated with the increase in human population. Zisweiler (1967) names the following specific causes: direct extermination from hunting for meat, eggs, hides, furs, and feathers, souvenirs, superstition, trade or research, trophies; indirect extermination by destruction of natural vegetation, drainage of wetlands, ravaged water, air and water pollution, radiation, traffic, domestic animal disease, and changed flora and fauna; introduction of competing animals.

In light of current estimates of future human expansion and the destruction of tropical rain forest, it can safely be anticipated that many species will vanish in the next decade unless their habitats are conserved. In the past two centuries at least 101 bird and 46 mammal species have become extinct because of human intervention (Zisweiler, 1967). The Red Data Books (IUCN) provide an extensive analysis of mammals, reptiles, amphibians, and fishes in various degrees of endangerment, and a comprehensive review on amphibians and reptiles has been compiled by R. E. Ashton (Edwards and Pisani, 1976). The Fish and Wildlife Service, U.S. Department of the Interior, issues a monthly bulletin whose purpose is to keep the public informed of the rapidly changing situation. The following listing is taken from the February 1977 issue of the bulletin (Fish and Wildlife Service, 1977):

Category	*U.S.*	*Foreign*	*Total*
NUMBER OF ENDANGERED SPECIES			
Mammals	36	227	263
Birds	66	144	210
Reptiles	8	46	54
Amphibians	4	9	13
Fishes	30	10	40
Snails	0	1	1
Clams	22	2	24
Insects	6	0	6
Total	172	439	611
NUMBER OF THREATENED SPECIES			
Mammals	2	17	19
Birds	1	0	1
Reptiles	1	0	1
Amphibians	1	0	1
Fishes	4	0	4
Insects	2	0	2
Total	11	17	28

The reproduction of vanishing species has become a self-imposed duty in most major zoos and aquariums. There are formidable problems. Only a small proportion of certain species will breed in captivity. Thus there is very little whale reproduction in captivity, although it is feasible (Ridgway and Benirschke, 1977). Virtually no bats are bred in zoos, and only about 10 percent of captive reptiles propagate. Hummingbirds and penguins are notoriously difficult to breed.

For many endangered or threatened species, captive propagation is not feasible. The space and cost requirements for caging and care would be astronomical. Consequently, zoos and aquaria have accepted the challenge to propagate a selected number of severely threatened species that are also useful for exhibition. For many of these species, some basic biological information is needed (e.g., convenient techniques to determine sex in birds) (Olney, 1977). For some species (e.g., Przewalski's horse), genetic information is needed to prevent inbreeding depression. Others (e.g., Siberian tiger) are subject to diseases in captivity that need to be studied and eliminated.

Many aspects of reproductive physiology and communicable diseases present technical barriers to reproduction.

Zoos and aquaria are beginning to address many of these questions, and they need encouragement and support. Above all, restrictive legislation concerning endangered species and the transportation of specimens must be developed in consultation with these institutions; it must not become a further impediment to achieving reproduction in captivity.

Research

Many biomedical questions can hardly be addressed unless animal models are available. An understanding of some of the basic aspects of nature has come from the comparative study of animals--their genetics, diseases, and so on. Man has greatly profited from this study, particularly in medicine. There would have been little progress in virology and preventive vaccination against poliomyelitis without the availability of nonhuman primates. Other investigations of disease (e.g., slow virus brain diseases and tumor virus research) depend heavily on primates.

Much of this research is carried out in primate centers, but these centers suffer from drastic reduction in availability of animals from the wild. India reduced exportation in 1975 from 50,000 to 30,000 annually, South American countries have severely restricted exportation (ILAR, 1975), and Thailand has banned all primate exportation (Fitter, 1977). Certain species are unavailable for most research purposes, because of their endangered status or because supplies are unobtainable; examples are apes, golden marmosets and lemurs (Bridgewater, 1972). Hubbs and Bleby (1976), reporting to the Medical Research Council in England, find that "the laboratory primate is likely to be of rapidly growing importance in many fields of biomedical research." They continue:

> Thus, even if it were feasible on health grounds to go on using wild animals, their supply would decrease with this increasing demand. The main solution to the problems would appear to be the early commencement of breeding primates along the same lines as all other commonly used laboratory species. This solution is recommended by the World Health Organization.

Progressive zoos are doing just that for all species available to them. Although few have the financial resources to undertake this massive job, staff of most zoos are deeply concerned about conservation.

Aside from primates, biomedicine gains enormously from the models found by chance in zoos and aquaria. In the comparative genetics of mammals, e.g., the lowest chromosome number (6) is found in a deer, *Muntiacus muntjac*, of which perhaps only 10 exist in zoos at present (Benirschke, 1977). This species' nearest relative has a chromosome number of 46; thus it would be very useful for genetic studies.

Funds have not been generally available for biomedical research in zoos because many institutions lacked qualified investigators and facilities, because much of the proposed research was unacceptable to zoo officials on the argument that their specimens were in the endangered category, and because of public pressures. This situation is gradually changing (ILAR, 1975), but a vast amount of material goes unused. Cell strains for mutagenesis, virus, and research on aging could be obtained from these collections. Virology on exotic species is important for human medicine and agriculture but is totally unexploited. Reproductive biology could be studied without threat to the species.

It would be possible to build up a systematic collection of cells of all varieties (see also p. 77). The cells should be conserved for use in large cell banks. The same can be said for spermatozoa, serum, blood cells, and embryos. A vast resource is not being used, and in many instances the material will not be available in the future for study by more advanced techniques. Only one strain of blue whale cells now exists, and only a total 250 fibroblast strains from other zoo mammals. There is no funding for a systematic effort to collect materials from any zoo species. Yet we can hope that through proper preservation of the nuclear genome the genetic content can be studied at a later date when extinction has caught up with the species and when nuclear transfer may become feasible. Although these prospects were highly speculative at first, it has now been shown in mice that individual foreign cell lines can be introduced into the embryo to render it chimeric and that it will reproduce this genome in future generations. Thus we can anticipate that the collection of somatic cells from vanishing species would be useful for the re-creation of these species in the future. Systematic collection should be undertaken at this time.

There is also a great need for research into reproductive physiology, exsemination (Seager *et al.*, 1975), ovum transfer, and the like, and this research must be conducted in zoos and aquaria.

While primate centers concern themselves with the most "biomedically useful" species, many other threatened species of nonhuman primates "fall between the cracks" (e.g., langurs, pigmy chimpanzees, lemurs, and orangutans). This situation must not be allowed to continue.

Properly trained biologists are needed to participate in research on captive breeding and disease control. Public funds for such research are virtually unavilable unless the programs have a biomedical connotation. It is essential that the public, legislators, and federal funding agencies be educated as to the dire need for work in this area before the trend toward extinction is further advanced. If the importance of conservation research to human health were adequately recognized, appropriate funds could be channeled into the work.

There is a great need for zoos and aquaria to become affiliated with universities and to conduct research in reproductive biology, behavior, and disease control. What is done for domestic and laboratory species (e.g., artificial insemination and sperm and blastocyst freezing and implantation) should be done for all species reproducing in captivity. Importation of animals for exhibition and for educational purposes would be unnecessary if the basic biological factors in reproduction were understood. If needed research on reproduction in captivity could be conducted, there would be less cause for concern about the imminent extinction of such species as the Puerto Rico parrot (whose few remaining specimens have not even been sexed), the California condor, and the bald eagle.

Short (1976) has called attention to the possibility of finding future domestic stock among zoo species. It is possible that some of these species could be developed into meat animals. Candidates include eland, beisa oryx, and perhaps special breeds of wild sheep and pigs. Some might prove to be desirable as meat animals because of their food preferences, which are different from those of the domestic animals we now have.

Regulation and Coordination

Because not all species can be artificially conserved, choices must be made. Zoos and aquaria are prepared to participate in this effort; these matters are repeatedly discussed at national and international meetings and in relevant publications. But they face restrictive legislation, severe financial problems, and often ill-advised public pressures. Zoos are establishing priorities, and breeding consortia are being formed, but there is no overall plan to assure that maximum conservation occurs.

Whereas new journals seek to correct the lack of awareness among scientists, only the Fauna Protection Society and IUCN attempt to bring all available data together systematically, but even here they lack sufficient funds for this attempt. When, occasionally, broadly trained biologists are available, institutions seldom have funds to support them or the needed research. Moreover, a flurry of new laws and regulations have severely impeded captive breeding. While it is recognized that the promulgators have good intentions--and that many of the regulations have been highly effective--the regulations are so restrictive, or the processing of forms so laborious, that worthwhile efforts are hindered. Large zoos now require a special staff to deal with permits as required to effect simple breeding loans of endangered species in our own country. Without a special permit (which is itself difficult to get), it is illegal, for instance, to send a tissue culture of a stranded dead whale to Edinburgh, where the only DNA research with this type of tissue is going on.

There is little communication between government officials and the persons who do the actual breeding work or conduct the research into reproduction of endangered species. So complex is the legislation and regulation on these matters that the Society of Mammalogists saw fit to publish for its members a summary of the regulations, but warned that the report should not be used as a primary source of information because of constant revision and review (Genoway and Choate, 1976; ILAR News, 1976). The rationale for and evolution of these laws is dealt with in great detail by the Council on Environmental Quality (Bean, 1977).

Well-intentioned captive breeding programs suffer from overregulation, delays, and many senseless restrictions. Better avenues are needed for communication between regulatory offices and the centers for reproduction and research, so that captive breeding can be vigorously pursued. An important recent instance is the destruction by the USDA of thousands of quarantined birds, including even endangered species, when Newcastle disease was identified in a single specimen. Alternative ways to deal with this situation exist and must be implemented. Likewise, we have ways of ensuring freedom from hoof-and-mouth disease and hog cholera that are unlike those currently used.

The endangered-species program has as its primary objective restoring threatened or endangered species to abundance. It does so primarily by regulating export and import. A secondary objective is captive propagation in survival centers. Conservation of germplasm is implicit. The criteria for the selection of species under the Endangered Species Act of 1973 are:

> The Secretary shall by regulation determine whether any species is an endangered species or a threatened species because of any of the following factors:
> 1. The present or threatened destruction, modification, or curtailment of its habitat or range
> 2. Overutilization for commercial, sporting, scientific or educational purposes
> 3. Disease or predation
> 4. Inadequacy of existing regulatory mechanisms
> 5. Other natural or manmade factors affecting its continued existence

The actual listing is similar to that of the IUCN, although a number of exceptions exist, presumably in part the result of pressure on the Department from special-interest groups and of inadequate time for study. An international treaty, implemented in 1977, severely limits importation and exportation of wildlife.

The IUCN defines an "endangered species" as a taxon "in danger of extinction, the survival of which is unlikely if the causal factors now at work continue operating. These taxa are those where numbers have been reduced to a critically low level or the extent of their habitat has been drastically reduced so that they are deemed to be in immediate danger of extinction." The next critical level is "vulnerable," followed by "rare," "out of danger," and "indeterminate." The individual species are carefully assessed by various scientific groups and documented in the Red Data Books, which contain information on status, distribution, population, habitat, conservation measures taken or proposed, and remarks.

Although the United States designations are usually similar to those in the IUCN listing, they often differ, and when different almost always place the species in a more endangered category. Because the IUCN listing is well documented, its recommendations should be followed whenever possible by the United States, and reasons given when deviations are considered unavoidable. The Red Data Books of the IUCN are periodically revised, and special groups of experts (e.g., the Rhino group) are assigned responsibility for recommending changes.

Continuity, Security, and Technology

Zoos and aquaria need no new regulations to ensure their continuity or to provide security for any given species. What they need is encouragement and support. Their success as organizations depends on their success in captive reproduction, and they are, therefore, highly motivated to pursue research in this area. But they urgently need additional scientific expertise to deal with a multitude of problems.

There is virtually no broad-based attempt to identify the genetic compositon of captive species, and no assurance can be given that captive breeding will be successful and not lead quickly to inbreeding depression. More biological investigation is needed to characterize breeding groups and ensure meaningful exchange between gene pools. Although these questions have been addressed in two reports (ILAR, 1975; Martin, 1975), much more work is necessary in this area. Also, at present no specific responsibilities for such exchange--or, indeed, for acting as guardian for any species--are assigned to zoos or other institutions. Breeding consortia are being formed (e.g., for the gorilla), and the Trustees of the Arabian Oryx have assumed international responsibility for the maintenance of this species, extinct in the wild. The removal of restrictive legislation would help these motivated groups.

ORGANISMS FOR RESEARCH IN GENETICS

Stock Collections

Breeding stocks used in genetic research constitute a particularly important germplasm resource. Comparable stocks used in the development of agriculturally and industrially important products are treated elsewhere in this report, because they present different management problems. We are concerned here primarily with the materials assembled in the course of, and for use in, basic studies in biology and medicine.

It must be emphasized at the outset that these materials are extremely diverse, and are highly varied with respect to their usefulness and their degree of genetic definition. The most extensively studied organisms, such as *Drosophila melanogaster, Escherichia coli,* and *Neurospora sitophila,* are available in extensive collections containing many natural variants, selected mutants, and combinations of genes and chromosome arrangements useful for different purposes. When many investigators use the same or similar biological materials, provision is usually made for the maintenance of a stock center to serve the needs of that community and to effect savings in the storage of materials used only occasionally. Such groups of workers may also develop an information-exchange newsletter, to announce the availability of newly discovered strains, the isolation of interesting mutants, and the development of new techniques.

Many collections, however, do not represent a community effort and a shared responsibility but are the product of a single individual's research. Genetic studies on any organism begin, of course, upon the initiative of one or a few explorers. The initial efforts may lead to wider use of the organisms, but in only occasional instances does it lead to truly widespread use.

These organism-based studies are an integral and important component of our national research effort. The "domestication" of the fruit fly led to the first compelling demonstration of the role of chromosomes in heredity. Studies on *Neurospora* eventually gave the first clear insights into the genetic control of metabolism. Genetic analyses of coliform bacteria and their viruses prepared the way for the explosive developement of molecular biology after World War II. Even now explorations are under way to discover whether new organisms (algae, nematodes, or squash bugs)--or old organisms used in new ways--may open new eras in regulatory biology, developmental biology, or population analysis. These collections of more or less genetically defined materials, accumulated through much effort over many years, usually supported by public funds, are an important national resource and are the basis for continued exploration of life processes.

Exploratory efforts sometimes lead, as noted just above, to the establishment of an organism as a model system for the study of particular biological processes. But the value of the materials thus accumulated does not depend solely on their incorporation into a comprehensive model system. The study of pathologies in animal models, for example, is an increasingly important means of approaching human disease. If a genetic defect is found in a mouse or a hamster that mimics muscular dystrophy, diabetes mellitus, or cerebral palsy, the mutant strain may be of great interest in medical studies, quite aside from its value in a mouse or hamster genetic program. Although this use of genetic materials in another species to illuminate processes in man is a well-understood approach, it is but one example of the utility of the comparative approach in biology and medicine.

An instructive parallel can be drawn between the task of managing germplasm for a commercial agricultural community and that of managing the germplasm requirements of the biomedical research community. Organisms in nature are the equivalent of the wild populations of a crop plant. Collections of organisms maintained for commercial use--in the production of cheese, wine, or antibiotics, for example--or for exhibitors, consumers, or fanciers of fish, birds, or mammals, correspond to the folk or land varieties of a crop plant. The few generally recognized model research systems constitute the payoff system of the research effort, much as commercial varieties are the culmination of the geneflow from native flora into crop plants. Between the wild populations and folk varieties and the major model systems, there are many smaller collections of organisms, more or less well defined genetically, with potentialities as major model systems. Just as genetic uniformity is an economic advantage in a commercial crop, so too it is advantageous to use a few well-chosen experimental systems in concentrated and collaborative research. And just as genetic uniformity in agriculture carries with it vulnerability to altered conditions or uses, so too can overreliance on a few genetic models have potential dangers in research. Thus research collections of a wide variety of organisms provide the basis for new investigative directions in a vital biomedical community.

Experimental Mammals

Biomedical research depends heavily upon the availability of genetically defined animal stocks--usually, although not exclusively, experimental mammals--for increasing understanding of normal and abnormal growth, development, differentiation, function, and aging deterioration. Analyses that require planned matings or tampering with the life history can be done only under experimental conditions. Primates, mice, and rats are, and will continue to be, extensively used in paramedical research both because of their intrinsic suitability and because of the "added value" of accumulated knowledge resulting from previous experimentation with the same species, inbred strains, or mutants. For researchers to gain from the experience of past investigators, and from special stocks that they have created, it is necessary that a considerable variety of genetically controlled animal stocks be permanently maintained.

Primates Both Old-World and New-World primates are very important in medical research. Collection of primates from the wild, which are then imported into the United States, has until recently been the chief source of research primates. But the eventual need to breed all nonhuman primates for biomedical research is now accepted (Bermant and Lindburg, 1975). For comparison with humans, primates are especially important in studies involving the nervous system. (See also p. 55, *et seq.*) Several different primate species, and variant populations within the squirrel monkeys, provide excellent material for the study of atherosclerosis. The reproductive physiology of primates corresponds more closely with that of humans than does that of any other animal group.

Monkeys are slow to mature and have few offspring so that establishment and maintenance of breeding colonies is necessarily slow and expensive, but is nevertheless essential for future supply of primates for research. This solution for research primate supply is recommended by the World Health Organization.

Mice Laboratory mice are very important in biomedical research, largely because so many and diverse inbred strains and mutants are currently available. Inbred lines (derived from more than 20 successive brother-sister matings) tend to be very uniform, including having the same histocompatibility genes, the same isozymic forms of enzymes, and a tendency for the same types of pathological lesions. These mice, plus special congenic stocks in which specific alleles characteristically found in one inbred strain have been transferred to a different genetic background, are very widely used in immunogenetic and other immunological research. All mammalian species thus far studied have one major histocompatibility locus, similar to the human HLA, and numerous minor loci. More is known about effects and genetic fine structure of the major *H-2* complex locus of the mouse, which corresponds to human HLA, than about any other histocompatibility locus in any mammalian species. The complex has effects on immune responses as well as transplantation. Continued advances in immunology will be greatly helped by continued maintenance and availability of a number of special genetic stocks carrying recombinations within the *H-2* locus. Such recombinations happen very rarely, but each must be kept as a critical research tool. A great deal of research on T- and B-lymphocytes and their interactions uses special congenic mouse stocks.

A predictable proportion of the mice in any one inbred strain develop lung tumors, mammary tumors, lymphatic leukemia, Hodgkin's-like lesions, amyloidosis, plasma cell tumors, or other specific degenerations and thus provide valuable tools for cancer or aging research. The incidences can often be elevated by carcinogenic agents, thus providing test animals for experimental carcinogenesis or for screening of chemicals. They are also valuable for screening of potential mutagens. The use of appropriately sensitive strains, and of a variety of strains, is important for obtaining conclusive evidence.

Deleterious mutants that occur in mice, whether spontaneous or induced, provide valuable tools for study of constitutional diseases that correspond to, or are at least similar to, human hereditary diseases. These mutants, of which a very large number are known, are most valuable if they occur in otherwise genetically homogeneous inbred mice, since the availability of a single gene difference segregating against a homogeneous genetic background greatly facilitates analysis of the action of the mutant gene. Because each mutation is a rare event, it is important that it be recognized, preserved, characterized, and exploited for its bearing on medical problems.

Rats Laboratory rats have been widely used in research, especially in studies of growth, learning and behavior. Much of this research has been based on well-characterized noninbred stocks, but many recent studies are based on inbred rats. It seems likely that genetically homogeneous inbred rats will become increasingly important as research tools. Old rats from different inbred strains develop specific pathologic lesions, including important tumor types. Two contrasting rat inbred strains have been developed--one with low, the other with high, incidence of dental caries. Special rat stocks have been developed for study of hypertension--one that develops hypertension spontaneously, and one whose hypertension develops only with a salt-containing diet.

Other Species Important genetic susceptibilities have also been recognized in a variety of other mammalian species. Mutant rabbits provide models of chondrodystrophy, adrenal hyperplasia, hemolytic anemia associated with leukemia and scoliosis, among other diseases. Mutant alleles carried by both pigs and dogs cause von Willebrandt's disease. Other strains of dogs have congenital heart defects, similar to human anomalies; still others develop rheumatoid arthritis or hemophilia. When armadillos are infected with leprosy they develop lesions corresponding more closely to human diseases than those to be found in any other experimental mammal.

Here, as elsewhere, the full usefulness of scientific reports describing these (and other) constitutional diseases in animals rests on the stocks being available in the future. Recognition and long-term maintenance of mutants in species not otherwise widely used in biomedical research is an issue that merits careful attention.

Critical Issues

Inventory Perhaps the most pressing need in the management of research collections is their coordination. By their very nature, these collections are maintained by persons with different interests and little communication. Perhaps the first step toward responsible management is to make an inventory. The major stock centers for well-known model systems are readily identified, but most of the smaller research collections are known to and used by only a relatively small group of investigators. Moreover, many research workers--even though they are not responsible for a stock center--possess unique genetic materials; e.g., mutants collected for a particular purpose, specially constructed stocks, or wild strains brought in for comparisons. One must be concerned, therefore, with maintaining current data both on collections of uncommonly used species and on uncommon genetic variants of commonly studied species. The newsletters of the major systems serve these needs, but no comparable service is available for the other materials.

Evaluation Not only do we need a centralized inventory of the collections, but the collections must be evaluated. The curator of a collection is often not an appropriate judge of the value of his stocks, even though--or because--he is very much aware of their cost. The need for evaluation arises from the cost of perpetuating collections and the limited funds available for such things.

Continuity Because a large fraction of the research collections are maintained by a few persons, or only one, their continuity often depends on the health, fortunes, or competence of a single investigator. If the principal investigator becomes ill or goes unfunded or retires, the materials assembled over a considerable career may be discarded or fall under incompetent management and be allowed to deteriorate.

Security Preservation of collections competes with other aspects of research. The need for a fail-safe incubator, a back-up electrical supply system, or a duplicate storage facility must vie with the need for a new centrifuge or microscope. A curator may undervalue his collection, at least in its role as a public resource, or he may simply be unaware of the best methods of managing it. A system of inventory and evaluation should include advising curators about effective management practices.

Storage Technology Recent technological advances permit much more economical and secure storage of many kinds of biological materials. The precise application of these techniques to particular species or strains, however, often requires adjustment and trial, and the research laboratories associated with minor collections are often not equipped to adapt the methods to their materials. More investigation is needed in the principles and practice of germplasm storage.

Financial Support Financial support for collections of genetic stocks is varied. Support of individual stock centers ranges from solid-based grant or institutional support to no support other than the investigators' personal resources. Furthermore, governmental support for such centers has not kept pace with growth of research. In some instances wherein governmental support has been discontinued funds have been provided usually on a temporary basis--by educational institutions or another agency.

The lack of dependable support seems incongruous with the importance of many of these collections as national and international resources. The importance of the collections, and the need for funds for maintaining them, should be brought to the attention of the public and various funding agencies.

Yet we cannot maintain every stock center or resource. It is difficult to determine just which resources should be kept and which have least likelihood of being important for research in the future. The National Science Foundation has asked the Committee on Maintenance of Genetic Stocks of the Genetics Society of America to suggest guidelines for making these judgments.

Responsibility Genetic stock resources are for the most part maintained by people who have research interest in their collections and who have taken, for various reasons, the additional responsibility of propagating and disseminating stocks for their own use and that of colleagues. Under these circumstances, immediate availability of a variety of stocks is an experimental advantage to the stockkeeper. This and other advantages offset the burden of finding funds to support maintenance of the stocks.

The greatest hazard attendant to individual supervision of collections is lack of permanence. Upon the death or sudden departure of the key individual, decisions as to continuation may be made by scientists or administrators who have no special insight into the stocks or the research involving them. Stocks are too often discarded without due notification to scientists who need them.

The Committee on Maintenance of Genetic Stocks of the Genetics Society of America recommends that an advisory body be formed around each major stock center and that these advisors come from outside the institution or agency that harbors the stock. These advisors should be chosen because of their expertise and interest in the particular stocks, and would assume the responsibility of arranging for the continuation of the stocks in the event of the departure of the stockkeeper. These advisors could also help the stockkeeper in determining what materials to add or delete and in other matters pertaining to stock maintenance. Additional responsibilities could be delegated to the advisors, their nature depending on the needs of the stockkeeper and his institution.

ORGANISMS FOR RESEARCH IN FIELDS OTHER THAN GENETICS

Organisms collected from the wild are used in many types of research, including cell biology, developmental biology, molecular biology, neurophysiology, comparative endocrinology and physiology, ecology, ethology, and systematics and evolution.

Research in molecular biology has been done mainly on genetically defined organisms, but some important contributions have come from molecular studies of plants and animals from natural populations. These include the crystallographic studies of whale myoglobin, studies on gene reiteration and gene amplification in amphibians, and studies of genome organization in sea urchin embryos.

The same general statement can be made about cell biology. Research in this area is done both on laboratory cultures and on organisms collected from the wild, but some of the more important work (e.g., that on chromosome structure and function) has been done on chromosomes of species collected from nature. Chromosomes of nematodes, insects, amphibians, and angiosperms have been particularly valuable as objects of study.

Research in developmental biology (embryology) traditionally has been carried out with organisms collected from nature. In fact, most of the important discoveries in this field have been made on material so obtained. Our knowledge of fertilization, the morphogenetic organization of the early embryo, embryonic induction, cell-cell interactions in tissue and organ formation, hormonal control of development, and the role of the nucleus in development has been obtained mainly from studies on organisms collected from the wild.

Fields of inquiry such as comparative endocrinology and physiology, ecology, ethology, systematics and evolution are almost totally dependent on natural populations of animals and plants.

Organisms in Ecosystems

Preservation of a wide variety of relatively undisturbed natural and managed ecosystems plays an important part in making it possible to detect and measure change, so that intrinsic factors contributing to natural change can be distinguished from humanly induced changes. An ability to do this will mitigate the common weakness in "before and after" studies of effects of environmental changes (e.g., damming a river or releasing heated power-plant effluent into the marine environment). At the present time, assessments of the "before" condition may be unrepresentative and observed changes may well include some that would have occurred even without imposed environmental disturbance.

Three kinds of intrinsic changes occur in ecosystems:

(1) Cyclical--at times very dramatic (e.g., the large fluctuations in population abundance in certain species of small mammals), but seem often to be related in some way to long-term persistence of the population; (2) Successional--may be slower than cyclical changes and thereby give an impression of stability; ultimately, however, they result in the disappearance of certain species (in managed ecosystems an effort is often made to hold the successional sequence at a subclimax stage); and (3) Stochastic--unpredictable by definition; the resilience of many ecosystems permits a partial or complete recovery to the previously prevailing stage. Above and beyond these intrinsic changes are the effects of external influcences, chiefly those related to intensified human activity.

In order to assess the relative effects of these changes on natural and managed ecosystems, environmental surveillance techniques must be invoked. A variety of measures have been used. Surveillance may be undertaken at any organizational and trophic levels within an ecosystem, from the individual to communities. For example, the identities of species present can provide considerable information, if their biology and ecology are well understood. The realization that valuable environmental insights could be derived from a species list, provided tolerances of individual species and relationships between the species were better known, has led to the concept of indicator species. In practice, however, such species are usually highly opportunistic organisms that have short generation times, wide dispersal capabilities, and the ability to take advantage of disturbance by increasing their population size very rapidly. The sudden explosion of a pest species population is usually an indication of gross changes in the ecosystem. Early effects of pollution usually include the disappearance of rare species from the ecosystem, yet most environmental surveys and laboratory tests of pollutants cannot perceive or monitor these effects.

At the community level there are certain properties, such as diversity (the number of species and the distribution of individuals among species), that most nearly approach an ideal single measure in that they integrate the many individual and group contributions. Experienced biologists can make valuable assessments of data from single surveys by inspecting a list of species and their relative or absolute abundances. However, extensive surveys, in both space and time, produce such a mass of data that a summary, usually in the form of an index, must be substituted. Costs of sample collection, sorting, and identification are high compared with the cost of data analyses. It is therefore important to make the fullest possible use of the results, and different indices may profitably be used for different purposes. Hellawell (1977) has summarized the chief properties of a number of indices.

Marine Species

As noted earlier, the variability residing in populations of marine species may be distributed in a continuous or semicontinuous way over a large latitudinal gradient. Effort to understand the meaning of the total genetic variability for the survival of the species should therefore be intensified and a better understanding of the degree of interconnection and interdependence between semi-isolated populations sought. Marine and estuarine environments include a number of ecosystems characterized by extremely high levels of diversity, and many marine species are themselves highly genetically variable. It has already been argued that the only method of preserving these high levels of genetic diversity is to preserve natural ecosystems in sufficient number and variety, and of sufficient size, to maximize the diversity of forms that can be maintained, and that special attention should be given to the most diverse systems: the tropical coral reefs and the deep sea.

Considerable progress has been made in developing laboratory techniques to maintain marine species of flora and fauna (Kinne and Bulnheim, 1970; Smith and Chanley, 1975). Yet there are still many features of the life history and biology of even relatively well-known commercial species that are not well understood, and this leads to difficulties when the species are raised on a large scale under hatchery conditions or are "farmed" under natural conditions.

Research on the marine environment is conducted and funded by a large number of agencies and organizations having overlapping interests and priorities. In order to carry out the task of preserving the greatest possible diversity of germplasm, appropriate organizations must assume responsibility for inventorying species, classifying and studying ecosystems, designating areas that should be preserved, promoting basic research on the biology and ecology of marine species, promoting applied research on marine species, studying acute and chronic effects on organisms and ecosystems of a wide variety of pollutants, and long-term monitoring of trends and changes in the marine environment. At the present time each of these responsibilities is assumed by a number of the following: universities; private research organizations; commercial companies conducting environmental studies and aquaculture projects; National Science Foundation; National Oceanic and Atmospheric Administration (including National Marine Fisheries Service and Office of Sea Grants); National Institutes of Health; Department of Energy; Environmental Protection Agency; Office of Naval Research; Bureau of Land Management; U.S. Department of the Interior (for example, the Bureau of Indian Affairs funds certain aquaculture projects); U.S. Geological Survey; Council on Environmental Quality; and municipal, county, state, and regional authorities.

There is much reason to feel that this multiplicity of agencies should be condensed into a single authority; on the other hand it has been argued that there are certain valuable safeguards in having a number of agencies funding projects related to their special missions.

Procedures for funding research generates certain difficulties. For example, when the total amount of money available for research becomes limited, there is a tendency to shift the emphasis from basic to applied research, to short-term rather than long-term projects, and to work that is thought to promise quick payoff. Most of the agencies fund projects on a year-by-year basis; few research grants commit funds for periods greater than 2 years. Yet certain kinds of work require a more sustained effort and some assurance of funding for a longer period (e.g., long-term ecological measurements and genetic studies).

Again, overlapping authority permits important, but perhaps unfashionable, research to go unsupported because it is not the special responsibility or charge of a particular agency. One such area is taxonomy, where for many groups of organisms there is but one expert in the world, or perhaps none. Yet unless the species in question is correctly identified, access to the literature on the genetics, physiology, behavior, ecology, or evolution of that species is hindered and it is therefore not possible to gauge the reliability of the information. For example, many baseline environmental surveys are based on a taxonomically very weak inventory and classification. Thus, the future value of such studies as reference points is highly doubtful. Yet the amount of educational and research support for training students in taxonomy reflects a general lack of research support for professional taxonomists.

Other areas of research suffer not so much from general neglect as from rapid changes in agency responsibility. Studies in marine genetics constitute one such area. All agencies concerned with marine research seem to be skeptical about the intrinsic value of genetic studies. Primary responsibility for genetic studies has not been clearly expressed or allocated, rather it has been transferred from one agency to another several times.

Macroscopic Organisms in Culture

Organisms now carried in culture were in the past often harvested on a continuing basis from wild populations. The trend toward maintenance under controlled conditions has occurred for two reasons: first, man's impact on the habitats of these organisms is thought to have reduced the size of the available populations; and second, certain research benefits from a greater uniformity in the genetic background of the materials under study.

Certain classic organisms have long been used in fundamental studies in cell and developmental biology. One thinks particularly of amphibian eggs and embryos, and sea urchin eggs.

Amphibians In the United States many leopard frogs (*Rana pipiens*) are used in teaching laboratories, and many bullfrogs (*R. catesbeiana*) as food. Up to now, virtually all of these have been collected from nature. So long as their habitats remain intact, populations of these species withstand both extensive collecting and occasional natural reductions. For example, where a long Indian summer is followed by freezing rains, leopard frogs in Vermont may not migrate to Lake Champlain to hibernate and may incur large losses over the winter, but the population recovers completely in about 3 years (Nace, 1976). Also, some experiments have been performed in which the frog populations of given lakes were almost completely removed; again the populations recovered in about 3 years (Nace, 1976). Thus amphibian populations show a remarkable capacity to recover from extensive losses so long as their habitats are preserved.

This situation appears to have changed during the past several years. Losses and changes in habitat, and perhaps losses from excessive collecting, have brought about reduction in the supply of the commonly used species. Several measures may be taken to combat this trend: habitat preservation; enactment of laws governing the collection of amphibians; preservation of migration routes between foraging and hibernating areas (e.g., by constructing highway underpasses); and commercial culture of commonly used species.

No one has yet succeeded in large-scale commercial breeding of amphibians, although efforts are being made. G. W. Nace (personal communication) states that he has about 15,000 frogs in his amphibian facility. He and his associates have developed new population cages, feeding devices, nonliving foods, and better methods of carrying the animals through metamorphosis, all of which have led to improved efficiency and productivity. However, Nace estimates that he is still several years away from accomplishing a commercially feasible operation. D. D. Culley (Lousiana State University) seems to be in about the same situation.

Of all the anurans, *Xenopus* appears the easiest to culture. It is still available from natural populations in large numbers, but import restrictions may affect access in this country.

One of the simpler aspects of maintaining amphibian germplasm concerns genetic stocks. These stocks, important in several types of biological research, are maintained in a few stock centers and in research laboratories. The long-range objective is to ensure that mutant stocks are preserved. If stocks are lost, it might be very difficult or impossible to produce them again.

Amphibian colonies are usually maintained by natural breeding; occasionally artificial insemination is used. Methods for routine preservation of sperm, eggs, and early embryos by freezing are not available. The most commonly used species of amphibians produce large numbers of eggs at each spawning, and in captivity may be induced to spawn every 2 or 3 months. However, generation time is long, ranging from about 7 months to more than a year. For genetic studies it would obviously be desirable to search for amphibian species with generation times considerably shorter than this.

Sea Urchins Sea urchin eggs have also been used in fundamental studies in cell biology. Yet it was only a few years ago that the populations of *Arbacia punctulata* off the coast of New England declined dramatically. It was then apparent, first, that biologists did not fully understand the reasons for the decline and, second, that it would be difficult to substitute material from other species for comparable studies. Since then, other species of sea urchins have been exploited more heavily as a research resource, but even some of these populations (e.g., *Strongylocentrotus purpuratus*) may be threatened by commercial exploitation. This has led R. Hinegardner and others to search for reliable methods for culturing species of sea urchins under laboratory conditions. These techniques and others applicable to a variety of marine organisms will be summarized in a forthcoming report by the Committee on Marine Invertebrates, ILAR, National Research Council.

Other Species Other species of harvested organisms--the squid, several species of aplysiids, the lobster, and the horseshoe crab--have long provided the material for fundamental studies in neurobiology. Some of these continue to be obtained solely from wild populations, but the Division of Research Resources, National Institutes of Health, has funded studies on the culture and life-history attributes of squid and a number of closely related species of Pacific aplysiids.

Various other organisms are in specialized collections important to basic research. A few examples will suffice: *Caenorhabditis elegans* (behavior studies); butterflies, *Tribolium* (population biology); and *Chironomus* (gene regulation). Others are raised for toxicity testing of pollutants.

Guidelines for toxicity testing of pollutants (Stephan, 1975) recommend the use of large or relatively robust species, the primary criterion for this purpose being that they should be readily available or commercially important. Most such species are in fact obtained from commercial suppliers, and only in some species of fish are hatchery stocks, of known genetic backgrounds (for example, the fathead minnow), used in such tests. Some pollutant studies on marine invertebrates have used species that have been inbred over several generations, but in most cases little is known about the effects of inbreeding *per se*.

Studies of marine pollution have increasingly shown that the pelagic larval stages are likely to be highly sensitive and therefore of special value in studies of the effects of pollutants. However, the parents of the larvae are, in almost all cases, taken directly from wild populations; hence genetic differences in susceptibility to pollutants are not taken into account.

One broad-scale study, recently initiated by the Environmental Protection Agency, will use the mussel *Mytilus edulis* as a monitor of pollutants. This species is widely distributed along the coasts of Europe and the United States and the post-larval stages are sessile and relatively long-lived. Thus populations provide evidence of trends in the environment, and individuals act as indicators of environmental effects.

Microorganisms in Culture

Most of the cultures of microorganisms in the United States are in specialized collections assembled with a view to public-health needs, agricultural or industrial needs, or for use in research. It is often difficult to distinguish microbial collections of practical importance from basic research collections, as illustrated by the collections of algae and bacteria used in studies of photosynthesis. These may be assembled in governmental or industrial laboratories addressing specific practical problems or in university research laboratories studying mechanisms of photosynthesis at the molecular level. Much research of basic significance occurs in practical laboratories, and much work of practical value comes out of the basic research laboratories. The same can be said of collections of nitrogen-fixing organisms, where the results of basic research are applied promptly to the solution of practical problems of immediate concern to world agriculture. The collections of organisms used in basic medical research stand in a similar relationship to those used in medicine and public health, as do many of those collections used in basic biochemical, pharmacological, and physiological research.

A few research collections consist of specially constructed stocks or wild collections useful mainly to an individual investigator; some consist of large, well-cataloged resources valuable to a considerable community of scholars. The extremes pose no problems. The "personal" collections cannot ordinarily be maintained once the "person" disappears from the scene. The obviously useful public collections will surely be preserved by some means.

The crux of the problem lies with collections that fall between these two extremes, given limited funds for preservation. A central decision-making organization composed of qualified scientists representing broad areas of expertise is required so that priorities can be set and hard choices made. The responsibilities of such a group should include not only the selection and perpetuation of good collections but also the recognition and support of new collections of potentially significant organisms not presently backed by large numbers of users.

One laudable trend, which should be encouraged and supported, is the establishment of broadly specialized central collections such as have been set up in recent years by persons responding to national needs. The Algae Collection at the University of Texas, Austin, assembled by R. C. Starr and now supported by the National Science Foundation, is an important resource operating at an effective level.* The International Collection of Phytopathogenic Bacteria established at the University of California, Davis, by M. P. Starr is the result of another effort to make widely available a comprehensive collection in a broadly specialized field. Although an extensive and valuable collection has been assembled, this center is unable to respond to more than a small fraction of the requests received, because of insufficient operating funds (M. P. Starr, personal communication). It is further threatened by the impending retirement of its curator.

The collection of the Anaerobe Laboratory, located at the Virginia Polytechnic Institute and State University, is another example of a valuable, broadly specialized collection. Its some 22,000 strains are heavily utilized, both nationally and internationally, by laboratories involved in human medicine, veterinary medicine, food production, pharmaceutical production, and the production of energy by microorganisms.

A system that incorporates such collections, each in the hands of competent curators, could do much to reduce the inefficiency and redundancy of the present situation. After areas of coverage had been firmly established and widely recognized, and curatorial expertise and continuity had been assured, many small specialized collections

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*The Algae Collection is arranging to acquire the more valuable stocks from the collection of L. Provasoli, of the Haskins Institute and Yale University, who has retired. These include unique pure cultures of marine phytoflagellates and marine invertebrates (Provasoli, 1976).

could be eliminated or consolidated, and valuable stocks from small collections could be saved, when endangered, by placing them in the central collections.

The system of broadly specialized national culture collections for microorganisms of economic importance has been adopted in the United Kingdom and the Commonwealth nations. An effort to determine how well this system has worked out in those countries would be warranted.

Ciliated Protozoa

The ciliated protozoa suggest an informative case history in germplasm management. They do not represent a topic of sufficient magnitude for extensive consideration here, but they may represent a class of problems. About 6,000 species have been described, but the more intensively studied species are found to have been previously "underclassified" (Sonneborn, 1975; Nanney and McCoy, 1976). Little systematic attention has been given to many major groups. A conservative extrapolation suggests that the number of isolated gene pools is at least 10 times greater than the number of named species; it may be as much as 100 times greater.

Cell Cultures

Cell strains from a wide variety of animals are used in biomedical research. For example, until very recently many nonhuman primates were killed in order to obtain kidney cell cultures for propagation of viruses in vaccine production. Most mammalian cell lines are readily preserved (with adequate protective agents) in liquid nitrogen. Cells of animals that die in zoos and at importation points are sought by geneticists, those doing research on aging, and other scientists. Only one center seeks to collect cells for its own research program. About 250 strains of various species are on hand in a collection maintained by the San Diego Zoological Society. In a few other laboratories strains of a few species are collected, but with no commitment to continuance.

Almost all of these cells are mammalian. Ten cell lines of eight species of fish have been preserved for diagnostic purposes by the U.S. Fish and Wildlife Service's Eastern Fish Diseases Laboratory in Virginia, indicating that preservation of fish cells is feasible. No lines of bird cells are known to us.

A large repository of "human genetic mutant cells" is maintained by the Institute for Medical Research, Camden, New Jersey, sponsored by the National Institute of General Medical Sciences (NIGMS), NIH. It is composed of well-characterized genetic mutants of human disease, chromosomal errors, tumors, and the like and exists as frozen vials of cell strains or cell lines. The bank, operated by scientists, periodically publishes a complete list of its holdings (third edition, August 1976). It also publishes descriptions of strains in scientific journals. The strains are available for purchase by qualified scientists.

In addition, the American Association of Tissue Banks, Rockville, Maryland, had its inaugural meeting in May 1977. The purposes of the association are stated in its Constitution and Bylaws as follows:

> 1. To promote scientific and technical knowledge concerning the procurement, processing storage, transplantation and evaluation of cells, tissues and organs, for clinical and research uses; hereinafter the term "tissue" shall include cell products, cells, tissues and organs;
> 2. To encourage the voluntary donation of cells, tissues and organs for clinical or research purposes;
> 3. To make available through regional tissue bank programs a safe, adequate and economical supply of tissues and organs for both clinical and research purposes;
> 4. To inspect and certify repositories for cells, tissues and organs used for clinical or research purposes; and
> 5. To establish Codes and Standards for cells, tissue and organ preservation used for clinical or research purposes.

It may well be that this organization will ultimately coordinate the efforts of numerous scientists in the collection and safeguarding of cells and tissues.

The American Type Culture Collection is a grant- and fee-supported private organization that collects, stores, characterizes, and ships a wide variety of animal and human cells, viruses, microorganisms, and so on. Its first catalog of cell lines was issued in 1975. Few wild animal cell lines are available through this collection, whose input depends on donors. The cost of cells to investigators is considerable, and it is difficult to understand how it is decided which cells to collect and maintain.

7 CRYOBIOLOGICAL PRESERVATION

An alternative to preserving germplasm by maintaining reproducing colonies in the wild or in the laboratory is to preserve it in a dormant state. The effectiveness of such an approach depends on the variety of cells in which dormancy can be reversibly induced, and it depends on the economics and reliability of the preservation techniques and on the institutional arrangements that are used.

The traditional procedures for storing or banking germplasm in the dormant state have been low temperatures, low water content, or a combination of the two. They have been used widely to bank microorganisms, cattle sperm, plant seeds, and animal tissue-culture cell lines. Other methods have been described from time to time, such as preservation of fungi under mineral oil, but they are mostly applicable to only a relatively few organisms. Here we are concerned with a rather special aspect of preservation in the dormant state--induced long-term dormancy achieved by freezing, drying, or both. This state has been termed "cryptobiosis" by Keilen (1959) and defined by him as "the state of an organism when it shows no visible sign of life and when its metabolic activity becomes hardly measurable, or comes reversibly to a standstill."

Closely coupled with the process of preserving germplasm in a dormant state is the need to resuscitate it when needed. Resuscitation, of course, includes consideration of the scientific and technical aspects of thawing frozen cells and with the rehydration of dehydrated or freeze-dried cells. The term can be extended to include the eventual utilization of the preserved materials, e.g., preserved viviparous embryos require the existence of suitable foster mothers or of techniques that permit complete embryological development *in vitro*. Again, to use preserved plant-tissue cultures there must be techniques for inducing the regeneration of the full plant from somatic cells in culture.

CRYOBIOLOGICAL PRINCIPLES

Freezing

The preservation of cells by freezing subjects them to a number of sequential steps any one of which is potentially lethal. These steps are: (1) the collection of the material and, for animal cells and some plant materials, its transfer to media that generally must contain molar concentrations of nonphysiological protective solutes (e.g., glycerol or dimethyl sulfoxide); (2) freezing *per se* to temperatures below -130°C; (3) low-temperature storage; (4) warming and thawing; and (5) removal of the protective solute and return to normal physiological conditions.

Steps (2), (4), and (5) are most likely to induce injury. The cooling rate in step (2) is especially critical. Cells must be cooled at controlled rates, the numerical values of which can differ by as much as 1,000-fold, depending chiefly on the size of the cells and their permeability to water and on the protective additive used. The mechanistic effects of cooling rate are becoming better understood, i.e., too high a cooling rate results in death from the formation of ice crystals within cells and their growth during warming, whereas too low a cooling rate causes death from the chemical consequences of the concentration of solutes during freezing or from osmotic forces operating during cooling and subsequent warming (Mazur, 1970, 1977; Farrant *et al.*, 1977b; Meryman *et al.*, 1977).

The effects of step (4) depend in a rather complex way on the prior cooling rate. Injury during warming results from growth of intracellular ice if cooling has been fast enough to induce it; rapid thawing minimizes this effect. Injury during warming also results from osmotic forces originating from events that occurred during slow cooling, forces that seem to be minimized by slow warming. Osmotic forces similarly play a role in step (5), the removal of the protective solute.

Step (3), low-temperature storage, causes no difficulty if the storage temperature is sufficiently low. "Sufficiently low," in this context means at least below -130°C, the glass transition temperature of water, or preferably

-196°C, the temperature of liquid nitrogen. At -196°C, there is good reason to expect that most of a population of stored cells will remain viable and unchanged for centuries or, more likely, for a millennium. At -196°C (and probably at -130°C as well), no known, biologically relevant, thermally driven chemical reactions can occur. The only sources of damage are photophysical events: ionizations produced by background radiations and high-energy cosmic ray protons. It has been calculated that 200 years would have to elapse to accumulate sufficient radiation to kill 63 percent of early mouse oocytes, one of the more sensitive mammalian cells, and some 3,000-20,000 years would have to elapse to kill 60 percent-90 percent of a population of "average" mammalian cells (Mazur, 1976; Ashwood-Smith and Friedman, 1977). So far as the induction of mutations is concerned, because less than 1 percent of the spontaneous mutation rate at physiological temperatures is due to background radiation, at least in mouse germ cells (Russell, 1963), and because background radiation should be the only factor contributing to mutational alterations at -196°C, the mutation rate at that temperature should be considerably lower than the spontaneous rate, even though biological repair is precluded at -196°C (Mazur, 1976; Ashwood-Smith and Friedman, 1977).

These theoretical considerations are consistent with experimental evidence. Although biological decay at -196°C is occasionally claimed, the consensus is that these observations are the result of artifacts and that no decay occurs at -196°C. Experiments now in progress subject mouse embryos to 100 times background γ radiation at -196°C. As expected, no adverse effects have been detected after 2 years of irradiation (Lyon *et al.*, 1977). On the other hand, at temperatures above -80°C, there are many well-documented cases of rapid decline (days to months) in cell viability (Meryman, 1966a). Here, too, there are sound physical reasons for expecting such declines.

An important question regarding the use of low temperatures to preserve germplasm is the extent to which freezing either induces mutations or acts to select preexisting variants in the population. Although the question has not been studied adequately, available information indicates that mutagenesis is nil. There is not only direct experimental information on microorganisms (Ashwood-Smith and Friedman, 1977), but several experiments on the freezing of DNA itself have shown no discernible effects (Shikama, 1965; Ashwood-Smith *et al.*, 1972; Ashwood-Smith and Friedman, 1977).

Nor does freezing appear generally to select preexisting variants in a population, a conclusion based both on direct examination in bacteria (Ashwood-Smith and Friedman, 1977), and on crude "epidemiological" evidence from the experience of repositories of frozen cell lines and from the large-scale use of frozen bull spermatozoa in the cattle industry. There are, however, a few documented instances of selection. Wild-type T4 bacteriophage and an osmotic resistant mutant differ greatly in their response to cooling rate; and in a mixture of the two types, cooling rates can be adjusted so as preferentially to select the osmotic resistant form (Leibo and Mazur, 1970). Similarly, germinated spores of the fungus *Neurospora* are much more sensitive to freezing than are nongerminated spores. Accordingly, if a population of spores is suspended in minimal growth media and frozen, the freezing will select in favor of auxotrophic mutants that are incapable of germinating in the minimal medium (Leef and Gaertner, 1975). Some cell populations (e.g., mammalian marrow cells and white blood cells) are physiologically and developmentally heterogeneous and differ in their cooling rate optima sufficiently to permit the enrichment of a given type by freezing (Austin, 1973; Farrant *et al.*, 1977a). Selection of preexisting variants can be minimized, of course, if freezing techniques are such as to produce overall high survivals.

Finally, there is the question whether the processes in freezing cause nonheritable and nonlethal morphological and physiological changes, and whether the freezing of germ cells and early embryological stages has teratogenic effects. Although the issue has not been studied in depth, it is clear that: (1) in most cases cells or organisms that survive freezing are indistinguishable morphologically, physiologically, and biochemically from the normal population, and there are no reports of teratogenic effects from freezing of sperm or early embryos (Whittingham *et al.*, 1972; Maurer *et al.*, 1977); (2) on the other hand, there are some well-documented cases where freezing does induce nonlethal alterations. Frozen-thawed sperm, for example, at times retains motility while losing its ability to fertilize.

The chief concern in using freezing to preserve germplasm, therefore, is not the sheer maximum attainable length of low-temperature storage, nor the induction of genetic or physiological defects in the survivors, but is to understand the events in freezing well enough to allow desired cells to survive both cooling to -196°C and the later return to normal physiological temperatures and media.

Freeze-Drying and Dehydration from the Liquid State

Freeze-drying (lyophilizing) consists of first freezing cells and then removing frozen water from them at low temperatures by sublimation *in vacuo*. A major fraction of the cellular dehydration actually occurs during the initial freezing, as the liquid cell water is converted to ice. The chief difference between freezing and freeze-drying is the ultimate degree of dehydration. Freezing effectively removes the approximately 90 percent of the cell's total water content that is "unbound" but it does not remove the residual bound water. Freeze-drying removes most of the latter as well (Meryman, 1966b; Mazur, 1968). So also do processes involving dehydration from the liquid state at above zero temperatures.

This difference between freezing on the one hand and freezing-drying or dehydration from the liquid state on the other hand probably explains why the latter two processes are considerably more deleterious to living systems. Generally the only cells capable of withstanding freeze-drying are bacteria and the spores and other dormant forms of fungi that regularly withstand dehydration in nature. The ability to survive freeze-drying is often critically dependent on the final water content. Drying to too low a water content can be immediately lethal; drying to too high a final water content causes rapid killing during storage of the dried product.

An early rationale for freeze-drying was that long-term preservation of cells would obviate the need for refrigeration. But this is not so; storage of freeze-dried cells at 4°C to -18°C is required to prevent rather rapid decline in viability (Fry, 1966).

There are well-documented instances in bacteria and plant seeds in which freeze-drying and air-drying induce mutations and chromosomal aberrations (Roberts, 1975; Ashwood-Smith and Grant, 1976). Roberts (1975) has calculated that the mutations accumulating in dried barley seeds stored under conditions that have led to a 50 percent loss of viability are equivalent to those induced in fresh seeds by 10,000 rad of X-rays. There is also a potential danger of selection, since in many cases only small percentages of cells in a population survive dehydration.

Although mutagenesis and selection are potentially troublesome, a number of culture repositories routinely freeze-dry and store microorganisms without apparent problems of heritable differences.

It is entirely possible that procedures could be developed for successfully freeze-drying a broader spectrum of cells, but the outlook is much less encouraging than for freezing, per se. Pragmatically the only advantage of freeze-drying over freezing is that the former does not require exceedingly low temperatures for long-term storage. But the cost of long-term cryogenic storage of frozen cells is scarcely prohibitive, and it is certainly technologically feasible to design adequate sensors, alarms, and backup systems to detect and rectify failures in the primary refrigeration systems.

STATUS OF CRYOBIOLOGICAL PRESERVATION OF GERMPLASM

Germplasm can be preserved in four ways: (1) by preserving the whole plant or animal; (2) by preserving somatic cells derived from the plant or animal; (3) by preserving individual germ cells or their direct progenitors (e.g., sperm, ova, pollen, spermatocytes, or oocytes); and (4) by preserving embryos. Methods (2), (3), and (4) have in common the requirement that procedures must exist for reconverting the preserved cells into reproductively competent plans and animals.

Prokaryotes

Most bacteria can be frozen such that they have high enough survival rates to permit the establishment of subcultures from the thawed progeny (Mazur, 1966). Some blue-green algae survive freezing well; others do not (Holm-Hansen, 1963). The basis for the difference is not known.

Plants

Whole Plants Most reproductively competent mature plants do not survive freezing to -130°C or below. This is true of fungi, mosses, ferns, and tracheophytes (Levitt, 1956, 1964; Mazur, 1968, 1969; Weiser, 1970), but there are exceptions. Some higher plants undergo a hardening process in nature that permits them to survive temperatures as low as -60°C (Levitt, 1956, 1964; Weiser, 1970). Some vegetative fungi, such as yeast, and many algae can survive freezing to -196°C under appropriate conditions (Mazur, 1966, 1968).

Somatic Vegetative Cells The above statements on whole higher plants apply for the most part to the preservation of differentiated plant tissues, such as leaves, stems, and roots, although here there are numerous cases of hardened woody tissues surviving to -196°C (Burke *et al.*, 1976). In contrast, there have been recent reports of the successful freezing of cultures of plant-tissue cells in which procedures similar to those developed for animal-tissue cultures were used. To date, cultures from about 10 species of plants have been frozen with varying success (Dougall and Wetherell, 1974; Nag and Street, 1975; Bajag, 1976; Towill and Mazur, 1976; Seibert and Wetherbee, 1977). The potential for the preservation of germplasm lies in developing procedures for the generation of whole mature plants from apical meristems or other relatively undifferentiated somatic cells. Full regeneration from tissue culture can now be achieved in carrot and tobacco (Steeves and Sussex, 1972), and undoubtedly procedures for the freezing and regeneration of other species will follow in due course. But achieving broader success may not be easy; certain forms, for example, are exceedingly sensitive to freezing (Towill and Mazur, 1976).

Reproductive Bodies Spores, cysts, pollen, and seeds that withstand air-drying in nature usually withstand freezing, if they are air-dry at the time of freezing. Full hydration, or germination, prior to freezing often causes them to lose their resistance (Ching and Slabaugh, 1966; Mazur, 1968b). Some spores, pollen, and seeds have not been successfully frozen. This latter result may be correlated with their possessing high water content in nature and with their relatively low resistances to desiccation.

According to Roberts and Ellis (1977), the International Board for Plant Genetic Resources has recommended that most seeds be stored at -18°C. Roberts and Ellis present data for barley showing that 1 day's storage at +40°C equals about 200 days at -20°C. Although -20°C may also be a satisfactory storage temperature for seeds other than barley, it will be costly and time-consuming to establish this fact. From the fundamental cryobiological consideration for hydrated cells, -20°C is an undesirable temperature, at least far less desirable than ≤-100°C (Mazur, 1966). True, storage at ≤-100°C is far more expensive than storage at -20°C, but to the cost of the latter must be added the considerable costs of periodically regrowing stored seed (Weiser, personal communication).

Animals

Intact Organisms, Except Embryos Instances of intact animals being frozen are restricted mostly to the protozoans, although there are sporadic reports of successful freezing of metazoans (e.g., rotifers, nematodes, and insects) (Asahina, 1966; Koehler and Johnson, 1969; Haight *et al.*, 1975). No vertebrate has ever survived freezing to more than a few degrees below 0°C. Even among the protozoans there are numerous forms that either do not survive freezing (e.g., *Amoeba proteus*) (Mazur, 1966) or in which only small percentages survive even under optimal conditions (e.g., *Paramecium*) (Simon, 1971). The outlook for successful freezing of the larger, more complex metazoans is bleak, partly because they are composed of cells of widely differing characteristics and partly because increasing size itself introduces formidable barriers.

Somatic Cells Several types of individual cells (e.g., erythrocytes, lymphocytes, bone marrow, and myocardial cells) and a few organized tissues (cornea and skin) can be frozen and retain high viability. But most mammalian tissues and nearly all mature mammalian organs do not survive freezing to below -20°C (Pegg, 1970; Karow *et al.*, 1974). On the other hand, fetal organs (heart and pancreas) have recently been frozen successfully to below -75°C (Mazur *et al.*, 1976; Rajotte *et al.*, 1976), and there are encouraging developments in efforts to freeze mature organs.

Among the more successful examples of freezing in animal cells have been tissue-culture cells. Most lines can be frozen so as to yield high enough survivals after thawing to ensure successful subculture (Pegg, 1970; Coriell, 1976). At least two major repositories of frozen cell lines exist in this country: the American Type Culture Collection and the Human Genetic Mutant Cell Repository.

Unfortunately, the ability to preserve animal somatic cells does not provide a method for the preservation of animal germplasm in a form capable of reproducing itself. Except for a few instances in lower forms, it is not now possible to generate a complete animal from a collection of its somatic cells and, unlike plants, it is not at all clear when, if ever, it will be possible to do so.

Animal Germplasm In the foreseeable future, then, the preservation of animal germplasm by low-temperature storage will entail the preservation of gametes and early zygotes.

• The preservation of bull spermatoza in 1950 (Smith and Polge, 1950) is said by some to have initiated modern cryobiology, and frozen sperm from highly selected bulls is now used in the artificial insemination of a large percentage of dairy and beef cattle. The sperm of a wide variety of other mammals has also been preserved by freezing. Those species in which frozen sperm has 50 percent or more of the fertilizing capacity of normal sperm include: bull, boar, stallion, ram, goat, and probably man. Those in which more than 30 percent of the sperm remain motile after freezing, but in which the fertilizing capacity has not been tested include: camel, moose, deer, bison, llama, yak, monkey, bear, chinchilla, dog, bighorn, and other wild sheep (Graham, 1973). Chicken and turkey sperm is viable after freezing, but both motility and fertilizing capacity are rather low (20-30 percent) (Graham, 1976). For several species of trout and salmon, fertilizing capacity of frozen sperm has proven to be high; for cod, moderate. Carp sperm is motile after freezing but has lost its capacity to fertilize (Horton and Ott, 1976). We have little information on sperm from invertebrates.

Although there seem to be no fundamental obstacles to the freezing of sperm from species not yet studied, the task will not necessarily be simple or routine. Over 20 years elapsed between the successful freezing of human and bull sperm and the successful freezing of ram and boar sperm. Empirical techniques developed for the former did not work for the latter, and many modifications failed. Neither the cause of freezing injury nor the basis of protection by various solutes is well understood.

• The successful freezing of sperm in 1946-1949 led to attempts with mammalian ova. Success, first achieved for mouse embryos in 1972 (Whittingham *et al.*, 1972), derived from fundamental cryobiological and reproductive physiological information that had emerged in the preceding few years. Since 1972 embryos of rabbit, cattle, sheep, goat, and rat have been frozen and have yielded normal viable offspring when thawed and transferred to foster mothers (Muhlbock, 1976; Ciba, 1977). The Jackson Laboratory in the United States, the MRC Laboratory Animal Centre in the United Kingdom, and others are now beginning to use the technique as an approach to the long-term preservation of potentially valuable mutant lines of mice, especially those not used on a day-to-day or month-to-month basis.

Whether early embryos from mammals, other than those cited above, will now be frozen easily is uncertain. Early stages of cattle embryos (1 to approximately 64 cells, cannot yet be frozen successfully, and pig embryos will not even survive chilling to 0°C (Ciba, 1977).

We know of no instance where an ovum or embryo from animals with large yolk-bearing eggs has survived freezing. At least two reasons suggest themselves: (1) only in the last few years has the successful freezing of cell aggregates been achieved (the obstacle has been that the permeability of cells to water and to solutes has a major influence on their fate during freezing, and permeability in turn is influenced greatly by cell surface-to-volume ratio); and (2) oviparous eggs, having been exposed to nonhomeostatic conditions, seem to have evolved protective mechanisms, one of which is an outer layer that has exceedingly low permeability to water and solutes.

ISSUES MERITING FURTHER STUDY

In some cases the techniques empirically developed for sperm have worked satisfactorily for other cells (e.g., many animal-tissue culture cells) and are in use today. In other cases the original techniques did not work at all well for other cells, not even for sperm of other mammalian species, and the derivation of successful procedures has involved tedious empirical testing at various levels of the several variables involved. In still other cases both the original empirical techniques and subsequent empirical modifications have failed. In a few of these last cases (e.g., mammalian embryos) success finally has been achieved because partial understanding of the fundamental aspects of low-temperature biology emerged in parallel with empirical studies. In other cases the level of understanding is still too primitive to suffice. Success in realizing the potential of cryobiological preservation will depend in large measure on the skill with which the techniques are applied to a wide variety of species and cell types by investigators differing greatly in their interests and in their knowledge of cryobiology.

The following seem among the more important questions that need to be answered in order to expand the potential of low-temperature biological techniques.

- What are the physical-chemical causes of slow freezing injury (e.g., concentration of electrolytes, osmotic forces, and thermal effects)?
- Where do the critical lesions occur and what is their molecular nature? There is increasing evidence that

membranes are more sensitive to injury than other organelles but, except for chloroplast thylokoid membranes (Steponkus *et al.*, 1977), the nature of the lesions remains largely unknown.

● What is the molecular basis for the action by which certain solutes protect against freezing injury?

● What are the permeability characteristics--and temperature dependences of permeability--of the cells in question, both to water and to solutes? Knowledge of the permeability characteristics has already proved a powerful asset in quantitatively predicting the optimum approaches to the freezing of several biological systems (Mazur, 1970, 1977; Leibo, 1977).

● Can the "scale-up" problem be resolved or ameliorated? As noted above, this issue relates to the fact that water and solute permeation depends on the surface-to-volume ratios of cells or cell aggregates, and these ratios decrease and become increasingly troublesome with increasing cell size: The larger the cell, the more slowly it must be cooled to avoid intracellular ice; the more slowly it has to be cooled, the more critical becomes the need for high concentrations of appropriate protective solutes; but the larger the cell or cell aggregate, the longer it takes for the solute to permeate into the interior of the aggregate; and, finally, the higher the concentrations of additive within cells or within cell aggregates, the greater becomes the likelihood of chemical toxicity from the solute and the more formidable become the osmotic problems associated with its removal after thawing.

Apart from questions such as the above, which apply to cells in general, there are sets of questions related to particular classes of cells. Answers to these more specific questions may well contribute not only to achievement of low-temperature preservation of the particular cell types but also to an understanding of their fundamental biology. For example:

● Why do sperm or ova from different mammalian species differ so strikingly in their susceptibility to freezing or even to chilling injury? Why is this sometimes also the case for different stages of embryos or for closely related cells from the same species?

● Can ova and embryos from oviparous and ovoviviparous animals be successfully frozen? If so, they will probably have to be made reversibly much more permeable to solutes and water. Can this be achieved?

- Some higher plant cells seem distinctly more sensitive to freezing than animal cells. Does this sensitivity arise from the existence of a rigid cell wall? Is it associated with interactions between the cell wall and the protoplast? Or is it due to the presence of a large vacuole in many plant cells?

RESUSCITATION

Germplasm that is preserved by dehydration or low-temperature storage must, when needed, be converted into a form that can reproduce. This conversion, or resuscitation, raises a number of intriguing and significant questions.

Animal Germplasm

Ova and Embryos It should not be difficult to resuscitate frozen embryos of oviparous and ovoviviparous animals, provided ways can be found to freeze them successfully. But the resuscitation of ova and embryos from viviparous animals (some of which can be frozen now) is far more complex. The most direct approach is to transfer embryos to the reproductive tract of foster mothers. Successful transfer requires that the foster mother be in a suitable stage of pseudopregnancy; this in turn requires knowledge of the estrus or menstrual cycle of the foster mother. Although such information is available for most laboratory and domesticated species, much less is available for wild animals, even those in zoos.

Cases arise where a nation needs to obtain nonindigenous germplasm but is unable to do so because of restrictions on the exportation and importation of animals, a matter discussed elsewhere in this report. One route to importation of germplasm is to import frozen embryos. However, the importation of embryos will be of no value if, because of quarantine restrictions, there are no females of the species to serve as foster mothers. Similarly, low-temperature preservation of embryos has been suggested as a method for preserving the germplasm of certain endangered species. But again the preservation will not be helpful if there are no females of those species left to act as foster mothers.

Perhaps the least speculative of the candidate techniques is the rearing of embryos of the desired species in the uterus of a closely related genus or species. This approach necessitates an understanding of the nature of the interspecies barrier to *in vivo* embryological development. More speculative, but still in the realm of possibility, is *in vitro* embryological and fetal development. In the last few years progress has been made in achieving further and further *in vitro* development of implantation stages of early embryos (Hsu *et al.*, 1974), and the age at which fetuses can be carried to term outside the female reproductive tract is becoming earlier and earlier.

Forms Other Than Ova and Embryos In theory animal germplasm could be preserved through preservation of the intact mature animal and through preservation of its somatic cells. In both cases the reduction of theory to practice remains highly speculative at best, perhaps impossible. Although a few small primitive intact metazoans and a few insects can be frozen, the size and complexity of most metazoans preclude for the foreseeable future their surviving freezing and thawing. But many animal somatic cells can be easily frozen. What remains highly speculative is whether ways can be found to derive a whole animal from some of its component somatic cells.

Plant Germplasm

By contrast with animal systems, the problem of deriving a whole plant from samples of its somatic cells seems well on the way to solution in an increasing number of species. Because attempts to freeze plant-tissue cultures were initiated only recently, we are not sure whether the task will be difficult or simple. But since it has been achieved in several plant systems, it seems reasonable to assume that the obstacles will not be insurmountable. If the assumption is correct, the combination of the preservation of tissue cultures at low temperatures with the ability to generate whole plants from the thawed material should prove a powerful approach to the preservation of plant germplasm, especially where the task is to preserve germplasm of plants that do not form seeds or that form seeds which are unable to survive long periods of storage.

8 DATA MANAGEMENT

Data on conservation of genetic resources and on the genetic resources themselves have traditionally been the responsibility of the individual most immediately concerned. Little concerted effort has been made to organize, direct, collate, store, and use these data. Such an attitude has brought us to the point where, to a large extent, we do not know where they are, what their quality or quantity is, what confidence we can have in them, and how they fit into a larger scheme of knowledge about organisms.

Today some of the most valuable knowledge about genetic resources resides in the holdings of museums, herbaria, and similar institutions. It is sometimes difficult to retrieve data or information from these institutions. There is usually only one avenue to information on the stored resources, and that is through the names of the organisms. Should one wish to retrieve all the names of the plants for a certain region, for example, it would be very difficult to derive such a list from the museum or herbarium because of the single-entry nature of the information storage system, yet inherently these institutions possess very valuable data banks. A move to place these data in computerized storage and retrieval systems is under way. (The SELGEM system of the Smithsonian Institution and the TAXIR system used at the USDA Regional Plant Introduction Station at Washington State University, Pullman, are cases in point.) A key deterrent is lack of appreciation among biologists generally of the need for data management systems. It might be instructive to know how much time and money is invested in data management, in light of the casual attitudes many scientists have toward their principal product, data.

Data management is, or should be, an integral function associated with conservation of genetic resources. If we do not know what we have and what we are now doing, we can hardly know whether conservation is being accomplished or whether we are merely paying lip service to some general concept. We cannot show, by reference to firm data, that any particular organism is, or is not, being lost. Aside from conspicuous plants or animals, whose existence is usually within the range of man's everyday observations, we cannot say with certainty that this or that species is on the verge of extinction. Indeed, there have been several situations in which, after an organism had been thought extinct, it proved to be alive and well in some part of the world in which we had not known it existed. Perhaps the most famous example among plants is the dawn redwood (*Metasequoia glyptostroboides*). It was long cited as a fossil in the United States and therefore thought to be extinct, but it was found during World War II in China. Shortly afterward it was cultivated in many places around the world and is no longer considered even an endangered species.

The institutions that manage information best are those with the longest record of management: zoos, botanical gardens, museums, herbaria, and live-culture collections. It is important that a critical and powerful organization committed to data management be developed.

A number of outstanding agencies, public and private, maintain systems for storing and retrieving biological literature. They include the National Agricultural Library, the National Library of Medicine, BioSciences Information Service, and Chemical Abstracts. Many private organizations that serve as information sources cannot accept responsibility for management of raw data. Their financial arrangements require that they recover costs, and there is little assurance that the market for genetic resources raw data would be dependable enough to ensure a profit. Both the National Agricultural Library and the National Library of Medicine are responsible for keeping up with and disseminating information about the published literature in their respective disciplines. Very likely isolated works in pure biology do not show up in their storage and retrieval systems. In any case, these institutions would be unable to accept unpublished data on conservation of genetic resources. Such data are merely descriptions of the actual plants or animals that are maintained or conserved.

The critical data are those describing the what, where, who, when, and why of the conserved species and genotypes. Data about the organizations and individuals carrying on conservation work are also important. Both types of data should be continuously updated. Standards of description, such as those existing in chemistry and physics, are needed and should be developed, as are networks for exchange of data. Many institutions are without funds needed to update their system of recording, storing, retrieving, and transmitting data and materials.

Continuous research into data management for all types of biological data is required, including not only information storage and retrieval systems, per se, but also programs for determining diversity within species, such multivariate analysis such as clustering methods, and computer-aided graphics for mapping and contouring.

A first-class training program for students and research workers is needed, to ensure that they understand procedures and the significance of accurate observation and recording. Many new methods are available for gathering data--from remote sensing, to electron microscopy, to protein analyzers--but it is often difficult to use these effectively in the conservation of genetic resources.

A number of institutions have set out to automate their data storage and retrieval systems for systematic collections and will continue this work. At the moment there is little or no coordination among organizations. Computer programs are being used on several types of computers; opinions differ as to whether this or that program is best for the purpose. We are badly in need of agreement with respect to some minimal set of descriptors for the accessed materials. If the arguments could be directed toward establishing the proper descriptor set, it would help greatly. Most sophisticated computer-aided programs require a set of data that has been structured to fit in certain ways and is adaptable to different machines and differently structured data. Standardization of the data structures on magnetic tape would be very beneficial.

9 FINDINGS AND RECOMMENDATIONS

GENERAL

- The diversity of germplasms is an essential national resource.
- The diverse germplasms are currently represented in a wide variety of forms including: free-living organisms in a great diversity of natural habitats in terrestrial, marine, and freshwater ecosystems; economically important organisms, including field, vegetable, nursery, florist, fruit and nut crops; forests; drug plants; livestock; fisheries; industrial microorganisms and pathogenic bacteria; specialized collections in zoos, aquariums, and botanical gardens that preserve and study rare species; organisms used in research, including specialized genetic stocks.
- The value of these resources is being rapidly eroded by a variety of encroachments.
- Action must be taken soon to protect and maintain the remaining genetic diversity, because once lost, much of it can never be recovered.
- The National Academy of Sciences should appoint a continuing Committee on Preservation of Germplasm Resources that would provide general surveillance on germplasm maintenance, and recommend specific attention to current and potential threats to genetic diversity.
- New agencies should be established, or existing agencies charged, with the preservation of particular germplasm resources: ecosystems, individual species of recognized significance, and gene pools of special value.
- Funds should be provided to support these agencies and to train the personnel necessary for the maintenance of these essential resources.

NATURAL ECOSYSTEMS

- Preserve carefully chosen existing natural habitats. These habitats, developed through millions of years of evolution, contain a wealth of valuable material that we understand only dimly and that we must preserve to protect the future of mankind.
- Protect extensive areas of existing natural ecosystems. A national inventory of these ecosystems and their components must be developed. We do not even have complete inventories of species in each ecosystem, and our current incomplete understanding makes it impossible to predict results of species loss.
- Enact laws that are designed to preserve total environments intact, with less specific emphasis on the endangerment of individual species. By far the best site for conservation of endangered species is within their intact natural habitats.
- Study natural ecosystems more fully. A scheme must be devised to evaluate the uniqueness of each particular habitat and to estimate the minimum area required to support the maintenance of an adequate gene pool of each component species. This system must be designed to provide an intelligent basis for decisions on preservation and evaluation. A master plan should be developed for the maintenance of each protected area.
- Encourage provision of manpower for the study of natural ecosystems, training of ecologists, geneticists, and taxonomists.
- Establish a National Ecological Reserve Board, backed by State Boards charged with study of regional problems.
- Recognize that preservation of natural habitats is a supranational problem; that we should have special concern for preservation of tropical ecosystems.
- Give particular attention to two categories of land-based areas: endangered habitats, to be held as public trusts; threatened habitats.
- Provide special protection for freshwater natural ecosystems (springs, streams, lakes, rivers, and associated wetlands) because of the pressures upon them of agricultural, residential, and industrial use. Aquatic habitats must be intensively studied and characterized, with inventory of species. Certain critical watershed systems must be preserved as essential natural habitats.
- Expand study of marine ecosystems. Research on characterization of marine ecosystems, and inventory of

the component species of each system, is essential. Study of life-histories of component species is important. Research on methods for laboratory culture and on the genetics of marine species should be encouraged. Communication between investigators studying marine ecosystems and government agencies responsible for enforcing protection of our sea and shore resources must be improved.

COLLECTIONS OF ECONOMICALLY IMPORTANT ORGANISMS

- Reemphasize concern expressed in *Genetic Vulnerability of Major Crops,* and elsewhere, for the status of our major food and fiber crops. Most of these have been developed from plants introduced from indigenous agriculture in other parts of the world. The genetic underpinning of varieties currently grown in the United States is dangerously narrow, and these varieties have proved susceptible to changes in exposure to pathogens or in environmental conditions.
- Make permanent and expand the National Plant Genetic Resources Board, and encourage cooperation with the International Board for Plant Genetic Resources, established by the Consultative Group on International Agricultural Research. National and international collaboration in the exploration, collection, conservation, documentation, and use of plant resources must be established on a firm and permanent basis.
- Support the aims and activities of the National Plant Germplasm Committee to: (a) develop a plan of repositories for clonally propagated plants; (b) provide facilities, staff and funds for USDA Inter-regional and Regional Plant Introduction Stations; (c) establish a facility for growing genetic collections of tropical plants; (d) set criteria as to the duties and responsibilities of curators of specific genetic crop collections; (e) select such curators, provide for their support, and guarantee their succession in case of retirement or death; (f) characterize major collections, with computerized data on plant genetic collections; (g) identify and rectify gaps in major collections; and (h) support research on techniques for maintaining collections.
- Establish nationally organized programs in each community or natural grouping of commodities to provide gene flow from genetic resource to cultivar. This would involve collection, maintenance, characterization and use of plant genetic resources, including information on

taxonomy; cytology and cytogenetics and biochemistry; screening for useful qualities; determination of inheritance patterns; development of improved breeding stocks and commercial cultivars; and supply of seeds or other propagules to producers.

- Maintain areas of indigenous subsistence agriculture of the antecedents of major U.S. crops at their geographical sites of origin. This activity should be promoted in the immediate future, since areas of subsistence agriculture in lesser-developed countries are currently diminishing markedly, due to increased industrialization, incursion of roads, replacement by modern techniques and high-yielding strains, and increase in large-scale monoculture.
- Preserve and support valuable foreign genetic collections, such as South and Central American collections of varieties of *Zea mays*. They should be subsampled and duplicates put in the National Seed Storage Laboratory. In this effort it is very important to avoid false confidence as to permanence of present arrangements.
- Promote study and preservation of the genetic resources underlying export crops of the lesser-developed countries. This includes tea, coffee, cassava, rubber, teak, and other forest products.
- Expand research on continual maintenance of high genetic diversity in forest management, so that long-lived trees can adequately meet many environmental fluctuations during their life-histories.
- Establish large wooded areas as Forest Genetic Reserves, in which seed collection only, but not logging, would be allowed and encouraged in order to maintain ancestral tree types, and to ensure a broad genetic base for future selection. Maximum genetic diversity of forest trees should be preserved, as it is impossible to predict the future needs of commercial forestry. The possibility of allowing forest seed collection in certain wilderness areas, national forests, and national parks, should be explored.
- Develop an organized collection of crop pests and pathogens and provide identification service and advice to those working in agriculture.
- Preserve natural habitats of important drug plants, as this remains the best way of assuring future supply of currently used drug plants and potential sources of useful new drug species. Continued availability of drug plants is important to the United States, since now and in the foreseeable future some 25 percent of drug prescriptions contain one or more important plant-derived chemical constituents.

• Preserve rare breeds of livestock to provide a reservoir of unique genetic traits needed for crossbreeding and selection of experiments to provide pertinent new breeds in the United States. Genetic flexibility may be especially important if feeding patterns are substantially changed in the future to conserve grain supplies. These stocks are in danger of extinction, and many are found only outside of the United States. Importation, seriously impeded at present by legal restrictions stemming from fear of exotic disease, must be facilitated by cooperative international efforts involving both breeders and quarantine experts, to protect future food supply. Special attention must be given in the near future to importation of exotic breeds of sheep and swine.

• Preserve and study marine habitats and ecosystems, placing particular emphasis on the habitats of commercial species and ecosystems characterized by unusually high diversity. Inaugurate genetic studies on selected marine species, in search of strains that can meet alterations in ecosystems.

• Support research on genetics and aquaculture of marine invertebrates. The commercial importance of lobsters, shrimps, clams, scallops, and oysters indicates the desirability of developing methods for their aquaculture and improving the understanding of the biology of these species. This should include characterization of genetic stocks and laboratory breeding experiments. The United States lags far behind other developed countries in this regard.

ECONOMICALLY IMPORTANT MICROORGANISMS

• Continue U.S. cooperation with the World Health Organization in its efforts at the international level to conserve germplasm resources of microorganisms pathogenic to humans.

• Organize currently diverse collections of pathogenic microorganisms in the United States under some common aegis to facilitate exchange of information, to provide continuity of needed collections, and to assure the general availability of organisms in existing collections.

• Identify organizational and managerial obstacles to conservation of our national resources of microbial germplasm in existing collections. The goal is to preserve efficiently, safely and economically, valuable materials that represent a major social investment. At present there is inadequate information exchange, availability of materials, and continuity of preservation. A single mammoth public collection is probably not the most effective resolution of the issue.

- Establish a coordinating agency to identify major collections, assign responsibility for their preservation and general distribution, help recruit curators and assure their continuity, maintain quality by assembling advisory bodies and to identify, locate, and assure support of smaller unique collections.

COLLECTIONS IN BOTANICAL GARDENS, ARBORETA, ZOOS, AND AQUARIA

- Recognize and utilize the potentialities of botanical gardens, arboreta, zoos, and aquaria in the conservation of rare, threatened, and endangered species. These institutions provide supporting environments for rare species second only to those found in their native habitats, which often have become severely limited in extent or, at times, completely destroyed.
- Support, under international direction, an authoritative worldwide inventory of rare species, their distributions and population sizes. This official list should include rare and endangered marine and estuarine species, and should provide the documentary basis for assigning a species to a threatened or endangered category.
- Encourage and support activities of a central organization, such as the International Union for the Conservation of Nature, to coordinate the activities of societies seeking to conserve particular species; to ensure that all necessary conservation activities are undertaken; to encourage efforts to breed rare species in captivity, including exchanges between institutions; to establish priorities, with decisions arrived at by qualified scientists, relating to species conservation efforts; and to work for continuity in funding of these efforts.
- Encourage research on the genetics and reproductive biology of threatened species in zoos, aquaria, botanical gardens, and arboreta. Sperm, ova, embryos, cell strains, and tissues from vanishing species should be saved as future sources of information on their characteristics. In many cases, additional research will be needed to devise appropriate procedures. These efforts may well be greatly facilitated by the establishment of research associations between given zoos or botanical gardens and nearby universities.

• Develop alternative approaches to facilitate necessary research and yet fulfill the intent of current laws and regulations concerning endangered species. Research on rare, threatened, and endangered species is severely handicapped by the formidable administrative difficulties of complying with these regulations.

ORGANISMS FOR RESEARCH

Genetic Stocks and Centers

• Continue maintenance of essential genetically defined stocks of organisms, including collections of viruses and bacteria, protozoa, algae, fungi, higher plants, insects (particularly *Drosophila*), amphibia, birds, and experimental mammals. Each defined stock reflects considerable effort; the organisms themselves have value commensurate with their degree of genetic definition and with the scientific knowledge that has resulted from their use. Small collections, the product of one investigator's work, are especially vulnerable beyond the founder's retirement. Larger collections of widely used species are often organized into stock centers, so designed as to maintain a large and defined portion of known genetic variation to supply organisms, information, and service to many investigators.

• Assign responsibility, as curators, to appropriate concerned geneticists, who have detailed knowledge of and interest in the organisms in a particular collection. Scientific boards, with members having pertinent expertise, should be appointed to advise upon each genetic stock collection. Maintenance of specialized collections or centers is preferable to combination of many collections in a single institution.

• Encourage and adequately support the stock inventory, registry and evaluation program (Committee on Maintenance of Genetic Stocks) of the Genetics Society of America, to provide coordination of effort and to assure continuity, security, and reliability. Support by the National Science Foundation of important Genetic Stock Centers, and maintenance of germplasm resources through support from the NIH Division of Research Resources, is very helpful, and should be continued and expanded. Other agencies should consider adopting the policy of direct support of genetic stocks to assure their continuing availability, not necessarily tied to support of research that utilizes a particular stock.

• Establish an advisory group for Mammalian Genetic Stocks, to set priorities for continuation of threatened mutants; to establish liaison groups to facilitate effective exploitation of new mutants; to promote establishment of mutants on appropriate uniform genetic backgrounds; and to promote cooperation among agencies (including components of the National Institutes of Health) for the maintenance of critically needed stocks.

• Continue, expand, and diversify the very useful organizational and informational activities of the Institute of Laboratory Animal Resources (NRC).

Organisms Obtained from the Wild

• Promote and support experimentation to develop optimal methods for culture and reproduction of wild species in the laboratory. Repeated harvesting from the wild has been the chief source of such critically needed species as echinoderms, various amphibians, various aplysiid mollusks, and nonhuman primates. Laboratory culture will go far to assure future supply, avoid perturbations of natural habitats, and sharpen the utility of these research tools.

• Protect populations of nonhuman primates in the wild and establish effective breeding colonies in the United States. For each important species, research on reproductive physiology and nondestructive genetic analysis should be encouraged. Especially during the impending period of restricted supply, while breeding colonies are building slowly, nonhuman primates should be used only for the most essential research, and carefully organized multiple use of scarce animals should be encouraged.

CRYOBIOLOGICAL PRESERVATION

• Encourage and support extensive research on the freezing and resuscitation of cells. This effort must include funds to examine the fundamental aspects of the problems as well as for application of these fundamentals to the development of pragmatic methods suitable to specific cell types.

• Seek better approaches for bringing the necessary talents into juxtaposition appropriate to the strong interdisciplinary nature of research in cryobiology and its applications require.

- Seek experimental confirmation of the generally held view that low-temperature storage provides an optimum method for maintaining genetic constancy of specific stocks. Theory indicates that mutation rate at liquid nitrogen temperatures will be much lower than at room temperature.
- Provide adequate support and protection for collections of frozen semen or embryos to maintain rare varieties of livestock, of frozen seeds, or other fruiting bodies of rare plant varieties. These may be an excellent way of preserving genetic diversity needed in future selection experiments.

MANAGEMENT OF DATA

- Involve biological scientists in designing data collection schemes for both the conservation process and genetic resources, using as consultants individuals with systems training.
- Develop a critical and powerful system of data management to accompany each conservation effort. Responsibility for seeing that adequate original data are collected should continue to lie with the concerned institution or governmental agency (NIH, USDA, USDI).
- Establish an overall monitoring group, with relatively long-term membership, to coordinate activities and maintain communication between different conservation efforts, and to assume general responsibility for quality and completeness of coverage.
- Provide a training program for students and research workers to ensure understanding of suitable procedures and of the significance of accurate observation and recording.

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