Genetic Resources at Risk:
Scientific Issues, Technologies, and Funding Policies

Proceedings of a Symposium
American Association for the Advancement of Science
Annual Meeting • San Francisco • January 16, 1989

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Report No. 5
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Calvin O. Qualset
GENETIC RESOURCES AT RISK

PREFACE

Genetic resources at risk was the subject of a half-day symposium in San Francisco, California on 16 January 1989 at the annual AAAS meeting. The goal of the symposium was to bring this important issue to the attention of a broad community of scientists, educators, research administrators, and policy-makers. In biological research, as in no other scientific field, new research is built upon access to living organisms or cloned genes discovered in previous research. Loss of any of these biological entities—animals, plants, or microorganisms—can result in a slow-down in scientific progress or, in some cases, a blockage to progress. This was dramatically illustrated on 10 May 1989 when a fire at the Jackson Laboratory at Bar Harbor, Maine devastated the facilities and about one-half of the 1700 genetic stocks of mice maintained at that laboratory. Back-up foundation stocks were not affected, so individual strains were not completely lost. The majority of genetic resources collections in the USA is far more vulnerable than the above-mentioned mouse collection, yet human welfare and productivity profoundly depend on their existence.

Genetic resources are often considered to be living biological materials of present or potential value to human populations. However, now, more than ever before, it is apparent that all biological resources have value in sustaining the world’s human populations. For example, plants may harbor genes of immense value for human health care, but these genes will remain undetected until the proper assay method or financial resources are available. In the meantime, conservation of biological resources is essential to hold the gene resources in reserve for future use.

The symposium was planned to provide a broad view of genetic resources with selected examples. Obviously, this short symposium could not develop case studies for the full range of species. Noteworthy was the lack of treatment to microorganisms and issues surrounding the conservation and documentation of cloned genes. It is hoped, however, that this brief report will illustrate the main points of concern and urgency. We hope it provides a stimulus for further discussions and development of action plans—both for the conservation of genetic resources and for the creation of new institutional and financial frameworks to provide national and international security for biological resources.

We were very pleased that the speakers enthusiastically accepted their invited topics. Several agencies, including the National Science Foundation, the National Institutes of Health, and the US Department of Agriculture, have had major impact on the advancements of genet-
ics through grants for research and for maintenance of genetic stock collections. We had hoped to include a summary of how these agencies are attempting to cope with the increasing need to maintain genetic resources, but the invited speaker was unable at the last moment to participate.

The symposium was chaired by Robert W. Allard, Professor Emeritus, University of California. We are grateful for his guidance and contributions to the discussion. In addition to the speakers, the success of the symposium was assured by the dedication of Dr. Patrick E. McGuire and Ms. Roberta Hooker of the University of California Genetic Resources Conservation Program to organizational and editorial matters. Members of the AAAS annual meetings program staff were very efficient and cooperative.

Calvin O. Qualset
University of California, Davis

Kenneth J. Frey
Iowa State University, Ames

Program Organizers
Genetic Resources — A Broad Perspective

Genes, specific sequences of nucleotides in nucleic acids (DNA and RNA), are the biological units of heredity which are common in their chemical basis to all living organisms. These genes, the biological resources of the world, packaged in virus particles and in the cells of animals, plants, and microorganisms, are natural resources, just as are the soil, air, and water, to be both used and conserved wisely by human populations. The term genetic resources encompasses nucleotide sequences, specific genes, well-defined genotypes, individuals, populations, and species at levels of organization ranging from plasmids, organelles, viruses, cells, tissues, and microbes to whole plants, animals, and fungi.

Genetic resources is a term sometimes used in a narrow sense to represent a specific component of biological resources, but it actually comprises a continuum in which three distinct categories can be distinguished by the degree of human intervention in the evolution of the resources. The optimum means of conservation differs among the three types.

- Dynamic, evolving populations of species in their native environments; including wild relatives of domesticated species.
- Landraces of agriculturally important species of plants and animals, modified over time usually by unconscious human selection and intervention.
- Germplasm and genetic stocks ("manipulated" genetic resources) developed by breeding and selection, spontaneous and directed genetic and chromosomal mutation, or biotechnological innovation.

<table>
<thead>
<tr>
<th>Dynamic, evolving populations of species in their native environments; including wild relatives of domesticated species.</th>
<th>Conserved in situ by protection of habitat.</th>
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<tbody>
<tr>
<td>Landraces of agriculturally important species of plants and animals, modified over time usually by unconscious human selection and intervention.</td>
<td>Conserved in situ by protection of primitive agricultural systems or ex situ in gene banks.</td>
</tr>
<tr>
<td>Germplasm and genetic stocks (&quot;manipulated&quot; genetic resources) developed by breeding and selection, spontaneous and directed genetic and chromosomal mutation, or biotechnological innovation.</td>
<td>Conserved in special, controlled environments.</td>
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Human uses of genetic resources include their study by sampling for laboratory research and in native habitats to advance knowledge of the earth's biota; the domestication and breeding of plants, animals, and microorganisms for food, fiber, and energy; the creation of highly specific genotypes to serve as tools of medical, agricultural, industrial, and biological research and commerce; the genetic engineering of organisms designed to produce specific products; the harvest of individuals from native populations of a vast number of species of the world's fauna and flora for food, manufacturing, and recreational pursuits; and the stimulation of the human sense of place and aesthetics.
WHAT GENETIC AND GERMPLASM STOCKS ARE WORTH CONSERVING?

Major M. Goodman

Abstract: Essential genetic stocks range from world germplasm collections of our major crops to genetic stock collections of experimental species such as Drosophila melanogaster. Not every accession of every major and minor crop, nor even all genetic stocks of our most important food crops can be conserved indefinitely. Nor can every ecological niche be protected, even though they may be harboring potentially useful and important but nondomesticated species. Even in the absence of scientific criteria for selecting appropriate germplasm accessions and assembling appropriate experimental genetic stocks, germplasm and genetic stock curators must constantly make critical decisions upon which entries are to be conserved on a constant basis. This discussion provided a framework for assigning some priorities.

Major M. Goodman is currently William Neal Reynolds Distinguished University Professor of Crop Science, Statistics, Genetics, and Botany at North Carolina State University in Raleigh, North Carolina and a member of the US National Academy of Sciences. Dr. Goodman, a native of Johnston, Iowa, received his B.S. in Mathematics from Iowa State University and his M.S. and Ph.D. from North Carolina State University. After an NSF Postdoctoral at the Institute of Genetics, University of Sao Paulo, Piracicaba, S.P., Brazil, he joined the faculty of North Carolina State University in 1967. The initial thrust of his research was upon the application of multivariate statistics to the classification of the races of maize, including the use of isozyme allele frequencies. More recently, he has been involved in the rescue of maize genetic resources as director of a program to regenerate the national maize accessions held by Mexico, Colombia, and Peru, to store seed for backup of these accessions at the National Seed Storage Laboratory at Fort Collins, Colorado, and to place adequate samples for distribution at the Regional Plant Introduction Station at Ames, Iowa. In addition he has been involved in the evaluation of maize genetic resources and is attempting to develop a program for utilizing them on a practical basis in his present position in charge of the corn breeding program at North Carolina State University.
I appreciate the opportunity to address the possibilities of choosing appropriate collections for maintenance, especially considering the problems that we are faced with in maintaining these very large collections. Obviously progress from plant and animal breeding depends upon germplasm, and indirectly it also depends upon genetic stocks. There are some big differences between germplasm stocks and genetic stocks in terms of difficulty and expense of maintenance and in the degree of demand for samples.

Germplasm stocks, generally speaking, usually consist of farmer varieties, landrace accessions, and wild or weedy or feral relatives of economically important (or potentially important) plant and animal species. Germplasm stocks may be used in basic research, but their most frequent use is for applied research involving, at least conceptually, improved plant and animal lines, breeds, varieties, or hybrids. Progress from breeding, genetics, physiology, and biochemistry depends to a great deal upon basic research conducted using our genetic stocks. These are mutant stocks, translocation and inversion stocks, various marker stocks, linkage tester stocks, etc. The two sets of stocks (germplasm and genetic) have to be handled very differently and represent very different sorts of problems and very different sorts of potentials, but they are both extremely important. But ultimately, in either case, future forms of these stocks depend on available genetic variations.

Germplasm stocks of most of our major agricultural and horticultural crops are being used for both breeding and basic experimental work. Table 1 lists the currently most utilized germplasm collections, while Table 2 lists the most important genetic stock collections. Animal germplasm collections are much less centralized than plant collections, but exotic (imported) landrace stocks of cattle and swine are making important contributions to breeding programs. Major animal species are not generally used to establish genetic stocks (such as markers and mutations) in the way that maize and tomato have been. Mice and Drosophila serve as substitutes.

<table>
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<tr>
<th>Table 1. Germplasm Collections Being Used Extensively for Breeding and/or Experimental Work</th>
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<tbody>
<tr>
<td>Coffee</td>
</tr>
<tr>
<td>Conifers</td>
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<tr>
<td>Cotton</td>
</tr>
<tr>
<td>Fruits/Nuts</td>
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The use of the germplasm stocks listed in Table 1 varies greatly over time, depending upon the demands imposed by various disease and insect epidemics. Wheat has traditionally suffered periodic epidemics of both diseases and insects; thus, wheat germplasm stocks have been widely used for breeding. In contrast, maize germplasm stocks tend to be used as much for basic research as they are for applied research; maize has had many fewer widespread epidemics than wheat.
The uses of rice, potato, tomato, soybean, and sorghum germplasm for applied research is also extensive. For tomato and potato, use has even been made of wild relatives in breeding work, a practice that is extremely rare — virtually non-existent — with maize’s relatives (teosinte and tripsacum).

In the past, humankind depended on an estimated three- to five-thousand species of plants. That has a message to us because today we are using only several hundred species of plants. In fact, we are obtaining the most part of our caloric intake from roughly 15 species of plants. These include most importantly cereals, grain legumes, and potatoes. The future of humankind depends vitally on those particular stocks.

Within a given species, as plant breeding has progressed, we have begun depending on fewer and fewer cultivars. I can speak with authority on only one crop, and that is maize. In the New World there are something like 300 races of maize. But, in fact, the maize of commerce represents only six of those 300 races, and the maize of commerce of the United States represents only one. We are relying on the use of a very narrow germplasm base in the United States and in the world. Within that one maize race used in the US there were, in the early 1900s, thousands if not ten thousands of open-pollinated varieties of maize distributed all over the US Corn Belt. Essentially farmers or regions had their own group of varieties used for specialized purposes. With the large-scale development of plant breeding in the early 1900s and the development of inbred lines for use in hybrids, only a few of those open-pollinated varieties were widely used in corn breeding programs. In particular, only two or three made very substantial contributions to corn breeding. Many of our hybrids today are represented by crosses between lines derived from the open-pollinated variety Reid crossed with inbred lines derived from the open-pollinated variety Lancaster. It is virtually impossible to find a hybrid that lacks a parent from either Reid or Lancaster. There are about six inbred lines and their close relatives that are represented in a very high percentage of all US hybrids. I cannot give you an exact percentage because it is not documented, but it probably represents well in excess of 50% of the planted acreage.

Most crops are not hybrid crops and they do not depend on inbred lines, but the general story is true of virtually every crop. We have a wide diversity of genetic variability among primitive cultivars and historic cultivars. We have a much lower degree of diversity among our recent and prospective cultivars. Basically we are increasing productivity at the cost of variability; this is true of virtually every crop. It is also true of all our domestic animals. Furthermore, most of our breeding work consists of crosses of elite lines by elite lines. When things turn desperate, we search our more primitive materials for a source of disease or insect resistance, and then go through a back-
crossing scheme to backcross that insect or disease resistance into elite materials. For this purpose we badly need access to adequate germplasm collections, but this need does not really serve as sufficient motivation to broaden our germplasm banks.

Disease and insects show no signs of ceasing to evolve around cleverly designed defense mechanisms, whether they be plant resistance imposed by traditional breeding or pesticides engineered by Du Pont or Monsanto. While molecular genetics may temporarily change this, plant and animal breeders, who, after all, have practiced genetic engineering for a century now, recognize that most resistance is temporary. As a result, germplasm stocks in demand today are likely to remain in demand tomorrow. There are a number of germplasm collections which are widely used. Dr. Rick is going to speak to you next, I believe, about the extremely important collections of tomato that he maintains. In fact, tomato is one of our pleasanter stories in germplasm collection, maintenance, evaluation, and utilization. In addition, potato has an interesting story behind it. Those collections have been very well maintained, but for many other crops the status of germplasm accessions is much less pleasant a story.

Answering the question of which germplasm stocks are important to maintain is reasonably easy. We can quickly answer which ones are critical: most of those in Table 1. What we cannot do as easily is respond to the problem of which accessions and which geographic areas need sampling and how many accessions are actually needed. For that, more scientific information is needed than we often have available. That a choice must be made is reasonably obvious, and I would like to use an example, again from maize. A maize breeder in a given year can adequately maintain about 30 collections (500 plants per collection, 250 hand pollinations per collection, 100 good harvested ears per collection). Thus, in a lifetime a maize breeder can maintain about a thousand collections. In the New World alone there are about 30,000 maize accessions, and there are four scientists doing the work. A very small amount of arithmetic says that some choices have to be made.

There are excellent germplasm facilities available that allow us to keep seeds of most germplasm accessions for a very long time. However, if we are going to have active distribution from these facilities, then we must have some way of replenishing the seed. There are several methods of tackling this problem that lessen its impact. One is to make geographic composites. Another, in the case of maize, is to make racial composites. Another is to identify sets of core collections, i.e., representative collections that span the range of genetic and ecoge netic variability present, and to work more intensively with those collections. Thus, those collections would be distributed routinely in preference to a larger set of backup collections which would be held for essentially final insurance purposes rather than for routine distribution.

An important point needing to be made is that whenever these germplasm collections are sampled, only a very few, usually one to five
percent (often much less), meet the criteria that the particular investigator is looking for. Nevertheless, it is desirable for crop germplasm curators to select and emphasize a subset of accessions representing the diversity of a crop. It would be desirable to have identified and available sets of 25, 50, and 100 accessions, each set representing, as much as possible, the diversity of the crop, in order to respond to the most typical types of requests. Such requests usually begin, “Please send me the (10, 15, 20, 50, or 100) accessions of (maize, wheat, rice, etc.) which most encompass the variation of the crop.”

If it is impossible to evaluate, adapt, or in the worst of cases, regenerate all accessions, then first emphasis should be placed upon core collections. While composite populations which represent combinations of individual accessions, such as the racial composites in maize, are often helpful for screening for general utility of germplasm materials, maintenance of individual accessions is essential, both for plant breeding and for basic research. Most genes of special interest are important because of specific rare alleles or, worse, specific combinations of rare alleles; the common alleles have already been captured by breeders, geneticists, pathologists, etc. Studies by numerous population biologists, many trained at Davis, California, by our chair, Bob Allard, have clearly demonstrated that many, if not most, such rare alleles are present in a few accessions at high frequency. Thus, screening individual accessions has a very high probability of identifying rare alleles, even in cases where replicated tests are needed for identification. In composite populations, such rare alleles must first be reisolated by the establishment of inbred or semi-inbred lines before screening can even be initiated. In addition, there is a high probability of loss of rare alleles through sampling during composite formation and maintenance.

I would now like to talk about genetic stocks (Table 2) as opposed to the germplasm stocks. These are being used in essentially an exponentially increasing fashion, mostly by the people who are working in molecular genetics. There has been a fairly constant demand for these genetic stocks from classical geneticists over the years, but in the past few years there has been tremendous demand put upon these stocks by the molecular geneticists. The stocks which are most important are maize and Drosophila. More mapping and related genetic research have been done with these taxa than with any others. Numerous organisms used for genetic work have no practical utility whatsoever. Mice, Drosophila again, and jimson weed (Datura) come to mind, but they have all been important in the study of questions of inheritance, biochemistry, etc.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Maize</th>
<th>Pea</th>
<th>Tobacco</th>
<th>Table 2. Genetic Stock Collections Being Used Extensively</th>
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<tbody>
<tr>
<td>Barley</td>
<td>Mice</td>
<td>Petunia</td>
<td>Tomato</td>
<td></td>
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<tr>
<td>Drosophila</td>
<td>Paramecia</td>
<td>Rat</td>
<td>Wheat</td>
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Gene stocks...
These genetic stock collections are of increasing importance. The public stock collections have proved to be inadequate, so a number of private companies have actually had to start their own private stock collections to supplement the public ones. Integrated genetic maps are badly needed and not available. The University of Missouri is attempting to mitigate this in the case of corn by attempting to integrate the RFLP maps with the morphological marker maps, but this is a long, tedious, and expensive process.

One of the problems with maintaining these genetic stocks is that the viability varies greatly from stock to stock. In addition, taking care of these stocks requires constant expertise. Every season they are grown they need expert attention. Many of them cannot be maintained in a homozygous condition. Virtually all of the genetic stocks are very high risk stocks. A season or two of a collection in the hands of the uninterested or the marginally competent may result in the instantaneous loss of stocks accumulated painstakingly since the 1930s.

The next question I would like to address is which genetic stocks are most at risk: they are not the ones that you might expect. In fact, the stocks that are most at risk are the ones that have been assembled by a single individual or by a single organization with no apparent person to take over once that individual retires. There is even some question in my mind as to what will happen to Charley Rick’s stocks once they are no longer under his care.

Examples abound of stocks that have been lost or endangered because of the retirement of leading workers in the field. Perhaps the most important set that has ever been lost as a result of a loss of a team leader were the stocks that were assembled in the 1920s and 1930s by the Russian expeditions of Vavilov. When he fell, virtually all of those stocks were lost.

Another thought which I would like to develop is that a germplasm system which merely acquires material and does not have facilities for evaluation and utilization is really not a system at all. In fact, many of the national germplasm banks that have been set up around the world fall into this category. Unfortunately, even some of the collections that we “maintain” (i.e., have acquired) in the states, fall into this category, because we really do not have facilities for regeneration or distribution, particularly for tropically adapted plants requiring short-day growing conditions. In many cases, I would maintain that seed banks holding such collections are really seed morgues. What goes in, is not going to come out alive. If we cannot regenerate and evaluate or adapt the existing collections, despite the clear need to do so, the obvious, but much less desirable, alternative is to drop back to a set of core collections which would be feasible to handle.

Without means of regeneration, evaluation, and utilization, we are fighting various battles, nationally and internationally, to acquire still more germplasm in a war that we long ago lost. Probably the only instances we have won are in the cases of rice, potatoes, and tomatoes.
For virtually every other major crop, we are just in various stages of losing.

The costs of not maintaining an adequate germplasm system are far greater than maintaining one. A single resistance factor in a major crop will more than pay for virtually all our agricultural research for a very long period of time. The set of wheat varieties which center around the variety Arthur have been documented as accounting for about $14 billion. Our annual public agricultural research costs are about $450 million. We are running our national germplasm system at somewhere around $20 million a year, which is about one tenth the cost of one B2 bomber. The national germplasm system cannot function adequately at less than twice its current funding levels. As a result, what we now have is a façade, not a system.

I would like to close with a quotation from Donald Duvick, Director of Research from Pioneer Hi-Bred International. He says, "Our national stinginess in collecting, storing, renewing, and describing our collections is inexcusable, not only in regard to our national obligations, but also in regard to our responsibility to the entire world."

DISCUSSION

Question: Does the complexity of the genomes have something to do with the size and number of accessions that need to be maintained?

Goodman: There is no indication that that is the case at the moment, but I am not sure that we know enough to answer the question well. There are some reasonably small genomes among our important cultivated plants, but I think with our present knowledge of genetics, we really rely more on ecogeographic diversity for enumerating the number of collections than we do on genetics. The simple reason is we cannot afford to do the kind of genetics needed to answer the questions. We are forced to rely on habitat diversity, and thus, we try to get an adequate sampling from diverse habitats. Only for a few crops, such as barley with the research that has come out of Bob Allard's lab, do we really have any sort of adequate measures of the genetic diversity available.

Question: You spoke primarily about plants, naturally, but my field is animal breeding and here we seem to have much greater genetic variability within strains and within breeds than you have in plants. What is the situation with animals?

Goodman: I am far from the world's expert on animal genetics and animal breeding, but the story I get from the people who are active in artificial insemination work is that the high rates of selection that are made for things like milk production and rates of gain are greatly reducing the number of effective sires that are going into each generation.
of animal breeding. I have heard figures that are so low I do not want to quote them here. These unbelievably low figures on the number of effective sires for the next generation will ultimately have a very drastic limitation on genetic diversity within breeds. One of the things we do in both plant and animal breeding is to keep demanding the very best things that are available. Farmers want the most productive crops and the most productive animals; they do a very good job of choosing the most productive material. This does have a tendency over time to narrow rapidly our germplasm base. I do know the poultry producers at one time encountered some fairly narrow germplasm bottlenecks. But I am really very unfamiliar with other animal situations.

Question: Do you have an idea of a solution?  
Goodman: Obviously the long-term solution is to broaden the germplasm base and use a wider diversity of animals. To some extent this has occurred in, say, the beef breeds of cattle and in the less fat breeds of swine. Materials have been introduced from other countries and broadened the germplasm base rather substantially. But this may be a one-time effect. I am not sure that we can repeatedly do this sort of thing. Usually when one brings in less highly selected material it costs fairly heavily in terms of economics of production. It takes a very long time to bring new genetic materials up to the level of production of the currently available elite materials. This is true both in plants and animals. It is a costly and time-consuming endeavor.

Question: Have you any idea what the Europeans are doing about this problem?  
Goodman: The Europeans have some very active work going on in germplasm that is specifically of interest to them, both in vegetables and temperate crops. My guess is that they may be slightly ahead of us in terms of the things that they have an active interest in. But that is more of a guess than knowledge.

Question: Do you think there ought to be on a commodity basis, nationally or internationally, some kind of a network that provides for assigning responsibility for maintaining germplasm diversity at least at some stage in the breeding process?  
Goodman: I think that is almost required. The commercial breeders are not going to do this. It is simply not in their best interest to spend lots of money on maintaining a very broad germplasm base. They are going to broaden the germplasm base only as economics dictate, and that means that the public sector is going to have to be the sector that maintains a very broad germplasm base. Private industry thinks of 15 years as long term. If you think that 15 years is long term, you have not made a cross between teosinte and corn with the hope of getting anything out of it.
Question: When you talk about narrowing the collections down to a core group, does that mean new discoveries or new collections will result in the elimination of something old from the core collection?

Goodman: No. The idea of core collections is not necessarily to eliminate materials. The idea would be to put entire sets of collections into essentially backup, long-term storage which would minimize the need for turnover. A set of core collections would be maintained for active distribution and routine use. Like most geneticists and plant breeders, I am the recipient of letters which say something like, “Send me the 30 most diverse collections of corn,” or “Send me the 50 most diverse collections of wheat.” We need to maintain sets of collections that answer such responses, because those are the most common, often constituting almost 99% of the requests. It is very rare that we need to go back to the entire set of collections; that happens to us only under the most dire of emergencies. Those entire sets can be kept under long-term storage for a sufficiently long period of time. We do not know how long we can keep collections under ideal storage conditions, perhaps several human lifetimes at the very least. This would reduce substantially the number of collections that need attention on an annual basis, and the core sets would identify specific sets of collections that need and merit substantial evaluation, characterization, adaptation, regeneration, etc.
PERSPECTIVES FROM PLANT GENETICS: THE TOMATO GENETICS STOCK CENTER

Charles M. Rick

Abstract: The Tomato Genetics Stock Center (TGSC) at the University of California at Davis maintains a collection of approximately 2,600 accessions of a) 13 interrelated Lycopersicon and Solanum species; b) genetic stocks: single and multiple gene marker lines, trisomics, translocations, and autotetraploids; and c) Latin American landraces and essential modern and vintage cultivars. The collection is internationally unique, few items being duplicated elsewhere. Many of the wild and cultivated accessions are now extinct in the native areas. The TGSC annually distributes 2,000 to 2,500 seed samples in response to approximately 200 requests from 100 to 130 investigators for a great variety of investigations. It is currently supported by the University of California and a grant from the USDA Agricultural Research Service and was formerly funded by the National Science Foundation. Regarded as a “curatorial collection,” the TGSC is not an integral part of the US National Plant Germplasm System. The main problems engendered by maintenance are: a) expertise to deal with the myriad requirements of such specialized germplasm and b) manpower to cope with the large seasonal demands for growing accessions, proper regeneration, inventory, documentation, and distribution. Heretofore, the TGSC has been inadequately supported to meet these demands. The California Genetic Resources Conservation Program has reviewed the status of TGSC and developed recommendations for long-term support.

Charles M. Rick received his B.S. degree from Pennsylvania State University in 1937 and his M.A. in 1938 and Ph.D. in 1940 from Harvard University. In 1940 he joined the University of California at Davis as Instructor and Junior Geneticist rising through academic ranks to Professor and Geneticist (1955) to the present as Professor Emeritus and curator of the Tomato Genetics Stock Center. He is also a member of the US National Academy of Sciences. In his early years Dr. Rick engaged in a variety of research, including genetics of sex determination and polyploidy in Asparagus, cytogenetics of interspecific Cichorium hybrids, and speciation in Nemophila. Discovery of male sterility and other causes of unfruitfulness led to development of a research program in tomatoes. Via aneuploidy, induced chromosomal deficiencies, and standard co-segrega-
tion methods, Rick and colleagues resolved the tomato linkage groups and related them to their respective chromosomes. He assisted in founding the Tomato Genetics Cooperative in 1949 and served as coordinator until 1971. In the 1960s his research focus shifted to natural relationships amongst tomato species: crossability, hybrid sterility, comparative variability vs mating systems, transmission genetics, and applications in the transfer of desired genes to the cultivated tomato. Dr. Rick has engaged in 15 major expeditions to collect tomato species in their native Andean region.

I am going to discuss the Tomato Genetics Stock Center which is a unit developed at the University of California at Davis. It grew like topsy with increasing research work being done on the tomato (*Lycopersicon esculentum*) and has gained some sort of official status in the last couple of decades. I will make reference to some other crop plants as we go along.

By way of introduction, I would like to say that the status of wild material and primitive cultivars is one that has changed since I first began working with tomato. We had our first trip to Perú in 1948 and at that time we collected quite a number of accessions, particularly of the primitive cultivars or landraces from Ecuador and the coast of Perú. Twenty years ago these disappeared, having been replaced by modern improved lines. If the collections had not been made at that earlier time, there would be nothing left of them. As far as the wild materials are concerned, pretty much the same story exists, especially for the coastal region. It is changing also in areas more to the interior, mostly as a result of ravages of goat herds. Again, we are very fortunate in having adequate samples of the wild material for a number of species from those zones.

In potatoes, the story is not quite so fortunate. Dr. Carlos Ochoa of the International Potato Center in Perú, probably the world's leading authority on potato germplasm, states that the greater portion of the old landraces of the Andean region are gone, even from a place like the Island of Chiloé in southern Chile which apparently figured so importantly in the development of domesticated potatoes for temperate zones. Many years ago, I saw and photographed examples of Professor César Vargas' potato collection, and I was quite intrigued by the variety of shapes, colors, forms, and uses that I was aware of at that time. Now the majority of these exist only in pictures. I had no idea at the time (1948) that the problem was going to be as imminent as it turned out to be.

I would like to say a word or two about the nature of genetic variation in the cultivated tomato. We have gradually become aware
that the tomato, especially as it existed until about mid-century, was vastly deficient in genetic variation. Based on indices of diversity calculated from isozyme data, one can compare genetic variation between populations and variation within populations for tomato and related species. For the more variable species, that is, ones that are largely outcrossing, the extent of genetic variability both within and between populations is vastly greater than that of the cultivated tomato.

Why a situation like this should exist is rather easy to understand. The commonly accepted ancestor of the cultivated tomato is *Lycopersicon esculentum* variety *cerasiforme*, the wild cherry tomato, which does not look very much different from the cherry tomatoes from the supermarket. It is native in the Andean region, and from there it has spread into many other parts of the world. In fact, it is a pantropical weed now. Unquestionably it moved up through Central America and the best approximation of the site of domestication is in the Mesoamerican area. After the discovery of America, it was transported to the Mediterranean region and gradually became accepted there. After more selection, it moved into northern Europe. At the end of the 18th century and throughout the 19th century, seeds of the stocks that were used at that time were transported back to North America, and they formed the basis of our breeding stock, up until about mid-century. Now you can well imagine that in all these moves, the ancestral forms were repeatedly reproduced in very small populations. These “bottleneck” population events as well as its natural self-pollination would have certainly tended to reduce genetic variation drastically, even after it was grown in Europe. Undoubtedly more artificial selection took place at many stages, further reducing genetic variation. It is rather no wonder that breeders have had difficulty up until about mid-century in deriving characters such as increased yield and disease resistance.

Progress in tomato improvement from mid-century to the present has been largely due to the introduction of desirable genes from exotic materials, the wild species and the primitive cultivars. This introduction has been very extensive; for example, resistance to at least 16 diseases has been bred from wild species. There are eight other species of *Lycopersicon*, all strictly wild. Only three of them have colored fruit; the rest of them have fruits that retain the green color at ripening.

The tomato is very cross-compatible with the species that have colored fruits. Crosses are somewhat more difficult with the other species, but with modern techniques, embryo rescue and so on, hybrids can be obtained. Thus, virtually all the *Lycopersicon* germplasm is available for breeders’ use in tomato improvement.

As an example, I will consider one of these species, *Lycopersicon peruvianum*, in detail. A typical habitat for it would be an irrigated valley in central Perú. The surrounding country is a total desert. The system of irrigation in such valleys is to tap water from a stream. The
water is irrigated through the fields; then runoff water is returned to a canal. A lot of this water eventually returns to the river source. This is a very democratic system for spreading soil-borne pathogens. It is no wonder that they have a lot of trouble with tomato culture in such valleys.

Rootknot nematode is one of these pests and is very prevalent throughout the irrigated valleys of Perú. Resistance to many strains of rootknot nematode has been found in *Lycopersicon peruvianum*. A single dominant gene, which confers this resistance, has been bred into tomato lines and has thus served very effectively in solving the nematode problem. The gene is located on chromosome 6 near the centromere. It has been denoted the \( Mi \) gene. Very fortunately, it is tightly linked with \( Aps-1 \), a gene coding for an acid phosphatase enzyme, so that the presence of this enzyme can actually be used as a selection criterion for nematode resistance.

Table 1 is a summary of all the disease resistances that have been detected in wild tomato species. The total number is 30 and the ones with asterisks, 16 of them, have already been bred into commercial cultivars. We have a vastly different picture here than the one that Dr. Goodman just presented. In maize they have been able to find all their germplasm requirements in old corn-belt stocks. Here with the genetically very depauperate situation of cultivated tomatoes a great deal has been done already by utilizing these wild sources.

In northern Argentina *Scrobipalpula*, a lepidopterous leaf miner, became a major problem. This insect can completely defoliate tomato plants and also attacks the fruit. Fortunately we do not have this insect in the US. In a test plot in the affected area of Argentina were planted one of the wild species, *Lycopersicon hirsutum*, cultivated tomato, and the \( F_1 \) hybrid between the two. I visited the area and was able to see the results. The degree of resistance was almost as great in the \( F_1 \) hybrid as it was in the wild species. There was nothing left of the cultivated tomato plants but the stems. This was another very neat demonstration of a desirable character that can be extracted from a wild species.

Many of the valuable attributes of wild species have been anticipated by noting the ecology of the regions where collections are made. One collection of cherry tomatoes was made from an obviously very wet place with a high water table. Indeed, resistance to water logging shows up in this collection. Drought resistance was found in *Lycopersicon chilense* collected in habitats where there was evidence of drought stress. The Atacama Desert in northern Chile never looked like a very promising area for plant collecting, but we had an inkling there was something we needed there. Lo and behold, we found a nightshade species, *Solanum rickii*, surviving. One plant bore a heavy load of ripe fruits, so we could get seeds. Other native species of plants of the area, including a very succulent one, had succumbed to the degree of drought there. In another area of northern Chile, plants of *Lycopersi-
con chilense were found in soil with a very thick layer of deposited salt, so it seems like a good bet we have a source of salinity resistance in this material. The highest elevation at which any of these species has been collected was in northern Chile at 3600 m just below Mt. Putre, in an area that no doubt gets frosts and severe freezes.

As an example of the extent of genetic diversity in wild species, I want to consider the species Lycopersicon peruvianum, which is distributed in Perú and northern Chile. We can recognize 35 to 40 races within the species. This is a matter of great interest as far as the use and maintenance of the collections is concerned. Within these races we are aware of still more variation. Then within individual accessions, there is an unbelievable amount of genetic variation, all of which must be taken into account in maintaining and utilizing this germplasm.

So far I have been talking mostly about species of the genus Lycopersicon, but there are four species of nightshade (genus Solanum) which are closely enough related to the tomato that they might eventually be used for tomato improvement. One of these, Solanum lycopersicoides, has been hybridized with tomato, and we are well along the way to the transfer of genes from it by conventional methods. Another species, Solanum rickii, looks rather similar to S. lycopersicoides, and we suspect it will not be very long before we can do the same thing with it. The other two species, S. juglandifolium and S. ochranthum, are more distantly related and utilization of them is something that is more on the horizon. The flowers of S. lycopersicoides have stamens or anthers that are white. Those of tomato and the other Lycopersicon species are yellow. White anthers turns out to be a monogenic dominant character. Progeny from a backcross that Joe DeVerna, Roger Chatelat, and I were able to develop showed a neat one-to-one segregation for yellow versus white anthers. Our experience with this character serves to illustrate that it is feasible to utilize this source of material.

Table 2 is a summary of the holdings of the Tomato Genetics Stock Center collection. Genetic stocks make up the major part of the collection. These include monogenic stocks; stocks that have a number of genes in them as linkage testers, for example; trisomics; tetraploids; allozyme stocks; and so on. Species accessions number around 1,000. These are accessions which are in sufficient supply to permit distributing them to investigators on request.

I would like now to indicate briefly our procedures for seed increase of the wild accessions. Increases of certain species have to be made in the greenhouse for a number of compelling reasons. We grow as large a population of each accession as we can. When they reach flowering we interpollinate them by collecting pollen from flowers of each plant and applying it to all members of the population. Usually two pollinations at weekly intervals are sufficient to provide all the fruit set that we need. Sometimes, extra measures are necessary. With Solanum juglandifolium, for example, we were able to obtain flowers
<table>
<thead>
<tr>
<th>Disease</th>
<th>Responsible Organism</th>
<th>Source of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collar rot</td>
<td><em>Alternaria solani</em></td>
<td><em>L. hirsutum</em>, <em>L. peruvianum</em>, <em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Leaf mold*</td>
<td><em>Cladosporium fulvum</em></td>
<td><em>L. esculentum var. cerasiforme</em></td>
</tr>
<tr>
<td>Anthracnose*</td>
<td><em>Colletotrichum coccodes</em></td>
<td><em>L. esculentum var. cerasiforme</em></td>
</tr>
<tr>
<td>Target leaf spot</td>
<td><em>Corynespora cassiicola</em></td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Didymella canker</td>
<td><em>Didymella lycopersici</em></td>
<td><em>L. hirsutum</em></td>
</tr>
<tr>
<td>Fusarium wilt*</td>
<td><em>Fusarium oxysporum</em> f. sp. <em>lycopersici</em></td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Phoma blight</td>
<td><em>Phoma andina</em></td>
<td><em>L. hirsutum</em></td>
</tr>
<tr>
<td>Late blight*</td>
<td><em>Phytophthora infestans</em></td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Phytophthora fruit rot</td>
<td><em>Phytophthora parasitica</em></td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Phytophthora root rot</td>
<td><em>Phytophthora parasitica</em></td>
<td><em>L. esculentum var. cerasiforme</em></td>
</tr>
<tr>
<td>Corky root*</td>
<td><em>Pyrenochaeta lycopersici</em></td>
<td><em>L. peruvianum</em></td>
</tr>
<tr>
<td>Septoria leaf spot*</td>
<td><em>Septoria lycopersici</em></td>
<td><em>L. esculentum var. cerasiforme</em>, <em>L. hirsutum</em>, <em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Gray leaf spot*</td>
<td><em>Stemphylium solani</em></td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Verticillium wilt*</td>
<td><em>Verticillium albo-atrum</em></td>
<td><em>L. esculentum var. cerasiforme</em></td>
</tr>
<tr>
<td>Dahlia wilt</td>
<td><em>Verticillium dahliae</em></td>
<td><em>L. peruvianum</em></td>
</tr>
<tr>
<td>Bacterial canker*</td>
<td><em>Clavibacter michiganese</em></td>
<td><em>L. hirsutum</em>, <em>L. peruvianum</em>, <em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Bacterial speck*</td>
<td><em>Pseudomonas tomato</em></td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Disease</td>
<td>Responsible Organism</td>
<td>Source of Resistance</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>----------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>BACTERIUM cont.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial spot</td>
<td><em>Xanthomonas vesicatoria</em></td>
<td><em>L. esculentum var. cerasiforme</em></td>
</tr>
<tr>
<td>Bacterial wilt*</td>
<td><em>Pseudomonas solanacearum</em></td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td><strong>NEMATODE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato cyst nematode</td>
<td><em>Globodera pallida</em></td>
<td><em>L. hirsutum</em></td>
</tr>
<tr>
<td>Sugarbeet nematode</td>
<td><em>Heterodera schachtii</em></td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Rootknot nematode*</td>
<td><em>Meloidogyne incognita</em></td>
<td><em>L. peruvianum</em></td>
</tr>
<tr>
<td><strong>VIRUS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber mosaic</td>
<td>Cucumber mosaic virus (CMV)</td>
<td>*L. peruvianum, S. lycopersicoides</td>
</tr>
<tr>
<td>Curly top*</td>
<td>Beet curly top virus (BCTV)</td>
<td><em>L. peruvianum</em></td>
</tr>
<tr>
<td>Veinbanding mosaic*</td>
<td>Potato virus Y (PVY)</td>
<td><em>L. esculentum var. cerasiforme</em></td>
</tr>
<tr>
<td>Spotted wilt*</td>
<td>Tomato spotted wilt virus (TSMV)</td>
<td><em>L. pimpinellifolium</em></td>
</tr>
<tr>
<td>Tobacco mosaic*</td>
<td>Tobacco mosaic virus (TMV)</td>
<td><em>L. peruvianum</em></td>
</tr>
<tr>
<td>Tomato yellow leaf curl</td>
<td>Tomato yellow leaf curl virus (TYLCV)</td>
<td><em>L. cheesmanii, L. hirsutum, L. peruvianum, L. pimpinellifolium</em></td>
</tr>
<tr>
<td><strong>NONPATHOGENIC DISORDERS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blossom end rot</td>
<td></td>
<td>all wild species</td>
</tr>
<tr>
<td>Silvering</td>
<td></td>
<td><em>L. cheesmanii, L. hirsutum, L. pennellii</em></td>
</tr>
</tbody>
</table>

*Genetic resistance from a wild species against this disease has been bred into a tomato cultivar.

only by grafting it onto tomato stocks. Our goal, of course, is to get as large a quantity of seeds as possible, which are put in long-term storage. Our vault at Davis, which maintains reasonably good atmospheric conditions, is where the bulk of our long-term storage materials are held. Duplicates of our accessions go to long-term storage at the National Seed Storage Laboratory at Fort Collins, Colorado. We maintain a working collection outside of the long-term storage conditions for distribution.

Table 2. Accessions held in the Tomato Genetics Stock Center listed by categories.

<table>
<thead>
<tr>
<th>TYPE</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild species</td>
<td>990</td>
</tr>
<tr>
<td>Monogenic stocks</td>
<td>730</td>
</tr>
<tr>
<td>Miscellaneous stocks</td>
<td>770</td>
</tr>
<tr>
<td>Allozyme markers; Chromosome markers; Miscellaneous combinations of markers; Linkage screening testers; Translocations; Autotetraploids; Trisomics; Modern and vintage cultivars; Latin American cultivars; Prebred lines (stress tolerance, chromosome substitutions, et al.)</td>
<td></td>
</tr>
<tr>
<td>Total listed accessions</td>
<td>2,490</td>
</tr>
<tr>
<td>Additional unassimilated accessions (mostly spontaneous and induced mutations)</td>
<td>250</td>
</tr>
<tr>
<td>Grand total</td>
<td>2,740</td>
</tr>
</tbody>
</table>

To conclude I would like to say a few things about the status of the Tomato Genetics Stock Center. We are currently supported by the Department of Vegetable Crops of the University of California at Davis and by a grant from the USDA Agricultural Research Service; additional support comes from the University of California Genetic Resources Conservation Program. This is enough to keep us going, but it is not adequate. We are dependent on student labor for a good deal of this work, and it leans rather heavily on me for direction. The Genetic Resources Conservation Program has taken on the responsibility of evaluating the collection* and probing solutions to the problems of adequate financing.

DISCUSSION

Question: What are the effects of local village culture on maintaining various wild species and various cultivars in Perú and in other South American countries? Have they given in to the newer cultivars developed by the international crop centers?

Rick: As with any other matter of economics, they do give in. You cannot blame them for it. Something that is higher yielding, that is going to improve their status, is very hard to resist. This is certainly happening. The threat of extinction is not so great as in other crops (e.g., potato), thanks to our extensive collections.

Question: What role do you see for in situ collections?

Rick: I am afraid the tomato is not a very good model for that. I mentioned the situation with Lycopersicon peruvianum. Are you going to have 35 reserves for that species alone? Then, what about all the other species? Considering this factor, the economy in the backcountry, and the problems that would be involved in protecting a reserve, I am rather pessimistic about the chances of successful in situ preserves.

Question: Is tomato unique in that respect?

Rick: I do not think so, because you have all kinds of other domesticated species in Perú. The same considerations would apply to Capsicum species (peppers), cucurbits, cotton, beans, and a long list of endemic crops and their wild progenitors.
THE DROSOPHILA STOCK AND RESOURCE CENTER: ITS ROLE IN BIOLOGICAL RESEARCH

Thomas C. Kaufman, Kathleen Matthews, and David Cribbs

Abstract. Drosophila has an extensive history as a tool in genetic research. This long-standing interest has resulted in Drosophila being perhaps the best genetically characterized eukaryote. Unfortunately, flies, unlike many other research organisms, cannot yet be maintained by cryogenic storage techniques. This fact has necessitated establishing stock centers for the safe keeping of mutation-bearing strains. The minimal function of these centers is to stock the most valuable germ line variants and to make these freely available. As an aid in this function, we have developed computerized stock lists which allow access to the collection. In addition to this minimal function the stock centers also 1) establish new mutant lines; 2) consult with investigators; and 3) are attentive to new technologies reported in the literature so that these may be incorporated into the collection. In light of this last goal, we have established a collection of P-element transposon stocks. These insertion elements serve as unique DNA markers and allow the ready cloning of adjacent genomic sequences. The Drosophila stock centers will continue to perform an essential function for the research community. However, this will only be so if an adequate funding level is maintained.

Thomas C. Kaufman, who presented this paper, received his B.A. from California State University at Northridge in 1967 and his Ph.D. from the University of Texas, Austin in 1970. He served as a postdoctoral fellow with support from the National Research Council of Canada at the University of British Columbia, Vancouver in 1971. He joined the faculty of Indiana University as Assistant Professor in 1976 where he is now Professor of Genetics. He has been a Senior Fellow in the Institute for Molecular and Cellular Biology at Indiana University since 1984. His research interests are the genetic, molecular, and developmental characterization of the member loci of the Antennapedia gene complex in Drosophila.
In the past few years, *Drosophila* has gone through a major resurgence and has now become a darling of the molecular biology community. Presently our service as a stock center is to the members of that research community. Since many of the users are new to the field, often the presence of the Center and its workings are accepted as the norm. However, there is a good deal going on behind the scenes. My comments today should shed some light on the way the Center works. I would like to divide my remarks into four basic sections, the history of the Stock Center, its current functions, a prospectus for the near future, and the nature of the funding we currently have.

There is a bit of numerology to keep in mind through all of this. One of the reasons *Drosophila* is prominent as a genetic research tool is that it has a small genome. Current genetic estimates are that there are somewhere between five- and ten-thousand mutable loci in that genome. Put another way, there are $5 \times 10^3$ loci that will mutate and produce a phenotype, either lethality or a visible phenotype such as eye color or wing shape. This number being relatively small, one stands a chance of actually mutationally saturating the genome and understanding the complete genetic repertoire of this higher eukaryote. Please keep that five- to ten-thousand number in mind.

**History of the Center**

The Stock Center began with the first collection of mutants established at the California Institute of Technology. This collection was brought by Thomas Hunt Morgan when he moved there from Columbia University with his students, Calvin Bridges and Alfred Sturtevant, in 1928. The collection remained at Cal Tech with Sturtevant and came under the direction of Professor E.B. Lewis, remaining there until 1987. The collection had to find a new home because Professor Lewis was approaching retirement and Cal Tech did not want to maintain it without him as director. At a *Drosophila* research conference a few years ago, I agreed to take on the directorship, and moved the stocks to Indiana University. Most of you are more familiar with Indiana University in the context of plant genetics; Marcus Rhodes, Ralph Cleland, and several other well known plant geneticists worked at IU. However, we should remember that H.J. Muller and Fernandus Payne established a tradition of *Drosophila* research there as well. Currently we have six fly labs and all the facilities for maintaining the collection. When Dr. Lewis transferred the fly stocks to IU in May of 1987, there were 1,500 mutant lines in the collection. Since that time we have expanded the number of mutant lines to roughly 3,500. We have also begun a collection of stocks containing P elements. At this time we have about 1,000 stocks of this type. Our long-term goal is to obtain a saturating set of these insertion elements, and I will elaborate on this point later. At present, the total number of mutant lines is about 4,500.

Despite its great genetic utility, one of the major problems with *Drosophila* is that there is no quiescent period in its life cycle. You
cannot freeze them. They do not make the equivalent of seeds. What this means, of course, is you have to keep the stocks reproducing continually. Maintaining the stock collection is therefore very labor intensive. This maintenance problem is compounded by the fact that we keep a duplicate set of the complete collection as a hedge against incubator failure. Each member mutant line is represented by three rotating vial cultures. This means that right now in the mutant collection, not counting the P-element collection, there are minimally 18,000 viable cultures going. Since these need to be changed every 12 to 15 days, we need to have several employees just to maintain the stocks in good condition. Based on current funding levels, the major function of the Stock Center has been simply to maintain this set of mutant lines.

At this point, I will present some information about content of the stock collection. The largest number of stocks contain point mutations that produce interesting phenotypes. The most familiar are the white eye mutations, curly wings, forked bristles, the kinds of things that bedevil undergraduate students in their first biology lab. We also have some rather esoteric things such as a mutant called shibire. These flies are perfectly normal at 22°C, but if you shift them to 29°C, they become paralyzed. Another example are the homoeotic mutations, like Antennapedia which causes legs rather than antennae to grow out of the head of the fly. We also have a large number of chromosomal rearrangements, balancer chromosomes, and interesting marker combinations for mapping and stock construction, very much like the Tomato Genetics Stock Center.

In addition to the genetic stocks of Drosophila melanogaster, we do keep some germplasm stocks. These exist in the form of wild flies caught from sundry locations around the world. For example, we have Swedish, Swiss, and Japanese fruit flies. However, there is a difficulty with putting too much importance on these. They are all Drosophila melanogaster which population geneticists have referred to as a "garbage pail" species. Drosophila melanogaster is so closely tied to human activity, it is hard to know whether the variation in these collections is due to the geographical origin of an individual population or whether the collection was brought in on somebody's garbage truck from another site.

With this large and growing collection we have found it necessary to utilize a commercially available database management system called Paradox. This system has greatly aided our ability to keep records and to disseminate information. We also are setting up a bulletin board on BITNET for Drosophila workers.

The other thing we do, of course, is send out stocks. In the first one-and-one-half years that the Stock Center has been at IU, we had 956 requests for stocks. Those requests resulted in our sending out 3,627 individual mutant lines to 24 different countries including the US.

We also are in the process of collecting and putting together valuable mutants and further expanding the stock collection. Current
research in *Drosophila* makes it important to increase the number of mutants that have interesting developmental defects. We have put together a set of chromosome deficiencies which will allow people to map readily mutations in the *Drosophila* genome and which saturates essentially 50% of that genome. We have also put together a set of stocks that we have obtained from Dr. William Engels at the University of Wisconsin which facilitates the ability to mobilize transposable elements within the genome of the fly. This mobilizer stock has resulted in the induction and recovery of a large number of new mutations that have very interesting effects on the anatomy and the development of the organism. Moreover, the presence of a transposon in the mutant locus makes the molecular cloning of these loci quite easy.

Finally, we are putting together what I referred to earlier as the P-element collection. These P elements are transposable entities within the *Drosophila* genome very much like the Ac-Ds system in maize. The molecular biology of P elements has proceeded to the point where they can actually be inserted into the genome mechanically by injecting P-element DNA into the developing embryo. Subsequently, one can recover individual insert lines in which there is transposable element DNA flanking a cotransformed genetic marker that allows you to detect the presence of the transposon in the genome. Using the Engel mobilization stocks, these individual insert lines can be jumped around the genome at will and moved to new positions. Our goal at the Stock Center is to assemble a set of P-element stocks such that there is a P-element insert every 10 to 20 kilobases in the genome. The reason for assembling this collection is that P-element DNA in these lines is unique in the genome and allows the molecular biologist access to every position in the genome. What that means is that the potential exists that the entire genome of this higher eukaryote could be cloned and characterized. This perhaps seems to be a rather lofty goal, but I think the important aspect of our function in the community is to put together this collection and have it ready.

A further important function of the Center lies in the construction of new stocks by juxtaposing novel mutant combinations and by combining multiple balancer chromosomes that are useful. One construction in particular we have just finished is the insertion of marker genes in which specific promoter elements drive the structural gene for \( \beta \)-galactosidase into balancer chromosomes. What these allow one to do is to distinguish histochemically different embryonic genotypes before any phenotype is revealed by a mutant lesion. Again, the ability to do this has come out of the recent advances in molecular biology combined with the genetic utility of *Drosophila*.

We also exist as a clearinghouse for information for the *Drosophila* research community. It turns out that a lot of the molecular biologists who are foraying into *Drosophila* for the first time need help with *Drosophila* husbandry and genetics. We can recommend the usefulness of certain stocks and the best culture conditions.
Our prospective for growth is limited by our carrying capacity in terms of the number of people we can accommodate in the laboratory and the number of incubator square feet available. With our current resources, we could handle approximately 5,000 mutant lines. As stated earlier, we now have 3,500. Also mentioned previously is the potential of identifying every locus in the genome of this organism (5,000 to 10,000); yet we do not have the potential to keep mutant alleles for every gene. We could, perhaps, keep one mutant allele of every locus, but we could not keep multiple alleles. However, if we kept point mutations at each locus, we could not maintain any aberration sets, any inversions, any multiple balancers, or any of the other interesting, useful stocks. This, of course, is a problem.

If the P-element Stock Center reaches its ultimate goal, we would like to have somewhere between 20 to 50 insertion points in every numbered segment of the polytene chromosomes. The optimum would be 50. There are 104 of those numbered polytene chromosome segments, so simple arithmetic tells you that this stock collection is going to be somewhere between two- and five-thousand stocks. If we assume the larger of the two numbers, that means that we would need a laboratory that would house 10,000 separate mutant lines. That would require much more of a facility than we presently have.

_Drosophila_ is burgeoning as a research tool. The number of people who call and the number of stocks we send out is enormous. We had no idea when we agreed to take on this Stock Center that the activity level would be like it is. We have had to install a telephone answering machine in the lab, due to the the number of inquiries and the fact that sometimes people in Europe do not pay attention to what time it is in Indiana.

The National Science Foundation (NSF) pays for the mutant stock collection inherited from Lewis. The funded amount is $100,000 a year. The University takes 47% of that for overhead, so actually we have only $53,000 annually. The Howard Hughes Institute is funding the P-element Stock Center. They have given us a sufficient amount of money to establish the Center, but there was a string attached to it. That is, they said they would buy the equipment, set up the P-element Stock Center, and fund it for two years only. There is no more money for longer maintenance. For long-term support for what I feel is an extremely valuable stock collection for this research organism, we are going to have to look to other sources for funding. We are at present negotiating with the NSF. If nothing comes from them, perhaps the National Institutes of Health (NIH), although I have been told the NIH is not in the habit of funding this sort of facility.
DISCUSSION

Question: Do you have any difficulty with USDA in shipping?

Kaufman: First of all, we do not write "fruit fly" on the packages because that immediately raises attention. We write, "Drosophila for research only. No cash value. Open immediately." Our only problem in terms of shipping has been to the Indian subcontinent where apparently overzealous customs officials like to open things. Usually people making a request, for example, from Australia or Canada, will send us special customs forms to use.

Question: How many people are involved in this; how much space is required?

Kaufman: Right now there are two curators, Dr. Kathleen Matthews and Dr. David Cribbs. Cribbs runs the P-element collection. Matthews runs the mutant collection which employs three half-time changers and one full-time media prep person. Cribbs runs the P-element collection which employs two technicians, one full-time and one half-time. The reason we can get away with so few (we do not have bottle washers, for example) is because there are six Drosophila research labs at Indiana University. Essentially the Stock Centers are being supplemented by that group of people. We are doing it on a shoestring.

Question: What would you consider to be an adequate level of funding in order to do everything you want to do the way it should be done?

Kaufman: Probably double the current figure; $200,000 per annum. There are two grants extant right now to develop cryogenic methods of keeping fly stocks. I do not know what progress has been made on those. I do not know that such a capability will necessarily be a panacea. The Jackson Lab's freezing facility for mouse embryos works well for some stocks but not so well for others, so cryopreservation is variable in its efficacy.

Question: Since Drosophila is not self-pollinated, a population produces new genotypes. How do you handle that for clientele that want to have the same thing they had before?

Kaufman: The lines that we keep are inbred, they are reproduced by sibling matings, so that works well. When we create new stocks, that is a problem. It has become a very big problem now with all of the interest from molecular biologists. The background in which a mutation has been recovered is extremely important because there is tremendous RFLP variability within the background of even this sort of ubiquitous species.
Question: You must have to handle orders very personally because you need to know what a person got before, if it is wanted again. You may not be able to supply it because of something critical some place down the line.

Kaufman: Correct. What happens is we get inbreeding depression. There is no way we can get around that. When we go through those bottlenecks, then we have to outcross to get the vigor back. When we do that we have to warn the recipients. The great thing about our database system is that we can keep not only all the genetic information, but also we have all the records of who got shipped what and when. We can keep very nice records of when outcrosses have taken place.

Question: Do you have any backup in other labs at other locations?

Kaufman: There are two other Drosophila melanogaster mutant stock centers. One of them is in Bowling Green, Ohio run by Ron Woodruff, also supported by the NSF. There is a European stock center in Umea, Sweden. Their collection is not as large as the two centers in the States, and they are having a big problem with funding. They are trying to get EMBO money, and I do not know that they are going to be able to get it. There is a species stock center also at Bowling Green which houses wonderful, strange, and esoteric different species of Drosophila.

Question: How do you decide what to put into a collection?

Kaufman: Right now we have not had to make any really critical decisions. What Kathy, Dave, and I do is go through the literature and look at the kinds of new things that are coming out. Recently we have just added the Tübingen collection of Nusslein-Volhard and stocks from Eric Weischaus. These are very large collections of important developmental mutations. Whenever we see new break points we try to get them, because break points are invaluable. It is terrible to see these things get lost. We also inherited the collection of Larry Sandler after his untimely death. There were some things in there that no one else had. We keep an eye on what is happening in the literature and the scientific obituary column which is the grimmer of the two.
FUNDING STRATEGIES FOR BIOLOGICAL RESOURCE CONSERVATION – EXPERIENCE FROM ENDANGERED SPECIES

Kurt Benirschke

Abstract: It has been virtually impossible to obtain public funds from the usual granting agencies to support a tissue bank for cells, DNA, or tissues of endangered species. This is true in spite of the National Academy of Sciences publication in 1978: “Conservation of Germplasm Resources - An Imperative.” The San Diego Zoo developed a Research Department in 1975 which is currently very active. At this institution, a “Frozen Zoo” was created for cell lines of all sorts of mammalian species, mostly endangered. This bank now contains over 1300 samples and is widely used for research and made available to qualified outsiders. It has been funded by the Zoological Society of San Diego through the foresight and wisdom of its Board of Trustees, who have made a firm commitment to its maintenance. Some monies are recovered from research grants. The National Institutes of Health initially participated in an institutional development grant. This bought some equipment. Nevertheless, the bulk of support has come from philanthropic donations, the “Kicks for Critters” campaign of my son, and the Zoological Society. It is essential that the public and granting agencies be educated to support such essential facilities.

Kurt Benirschke was born in Glückstadt, Germany. He was educated in various German universities and received his M.D. from Hamburg University in 1948. He immigrated to the United States in 1949. From 1951 to 1954, he continued his postgraduate education at various hospitals of Harvard Medical School. He was a Pathologist with the Boston Lying-in Hospital from 1955 until 1960. In 1960, he joined, as Chair, the Pathology Department of Dartmouth Medical School, Hanover, New Hampshire, where he remained until 1970. In that year, he joined the faculty of the University of California, San Diego Medical Center as Professor of Pathology and Reproductive Medicine where he continues today. From 1975 to 1986, he also served as Director of Research for the Zoological Society of San Diego.
This topic should be of concern to all of us as most of the large animals are going to die out very soon. My interest in conserving some of the genomes of large animals arose about 30 years ago when I lived in New Hampshire. I was interested in beginning karyotypic delineation of larger and usually very inaccessible animal species. One day I happened to collect a deer that had only six chromosomes while its closest relative had 46. We were so perplexed that we thought something had gotten into our culture and mutated it so as to fuse all the chromosomes to make six out of 46. It turned out actually that *Muntiacus muntjac*, the Indian muntjac, has only six large chromosomes, while the muntjac from Taiwan, *Muntiacus reevesi*, has 46. In order to do some comparative studies and find out more about similar things, I decided what we really needed to do is store some of these cultures. In the future, many of the comparative studies that we would like to do, particularly if new methodologies come along, will not be possible because we will not have a giraffe or muntjac or any of the other species. It turns out that for most large species, a blue whale, for example, there are no genomes you can access if you wish to have a culture or the chromosomes or anything else. This, unfortunately, is true of virtually all large mammals. With this in mind, I began freezing away fibroblast strains in a very systematic fashion. That is what I want to tell you about very briefly.

I did this initially in New Hampshire at Dartmouth, and when I moved to San Diego I took the cultures with me. They all died in a laboratory accident. The risk of accident is something that has not been talked about, but needs to be addressed in the future for collections such as these. If you have once gone through the trouble of collecting blue whales, gorillas, or orangutans with which you do research, you certainly do not want to have them die because the freezer sprung a leak and let the liquid nitrogen out over Easter.

I also want to address very briefly the problem of getting money for collections. This has been very difficult indeed; it is really what I was asked to speak about. In 1972 or 1973, I convinced the San Diego Zoo that they needed to have an independent research arm to do in-house research. They started in 1975 and I served there for 11 years doing this. During this time, I extracted from the Zoological Society a commitment to look after what has become an increasingly large and more valuable collection of strains of cells as well as spermatozoa and fertilized ova from animals. We have been successful at least locally in the San Diego area in making popular the notion of a frozen zoo. It is a very gross oversimplification, but it has convinced the public that we really need the money to do this. My son, as you may know, was a prominent San Diego Charger, and through the Kicks for Critters Program he was able to raise the sums necessary to maintain and build up the collection. The initial equipment money came from an equipment grant from the NIH obtained when I had a large NIH grant.

The zoo research activities have now been dubbed the Center for
Reproduction of Endangered Species (CRES), and I want to address what this collection consists of, who uses it, what sort of samples are being collected and shipped out, and what the cost of it is (Table 1). The frozen zoo basically is a repository of cell strains, fibroblasts largely, sometimes other cell types, that have been characterized for the most part. That is, almost all of them have had at least karyotypic analysis. They have been subcultured and a variety of vials has been prepared from them. They are relatively early cultures so that there are many further replications possible. They are kept in small vials in liquid nitrogen in duplicate tanks. I am desperately seeking a place where a second replicate collection could be housed away from possible earthquakes or fires. If a proposed animal genetic center at Davis becomes a reality, then we will be able to place a duplicate tank there. Its maintenance would require very little more than periodic checking and liquid nitrogen filling.

Table 1. Status of the “Frozen Zoo” of the San Diego Zoological Society

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
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<tbody>
<tr>
<td>1,350 mammalian cell strains</td>
<td></td>
</tr>
<tr>
<td>250 species of 11 orders</td>
<td></td>
</tr>
<tr>
<td>58 genera and 130 species of Artiodactyla</td>
<td></td>
</tr>
<tr>
<td>Permanent records include history of animal and ISIS number</td>
<td></td>
</tr>
<tr>
<td>Record system is computerized</td>
<td></td>
</tr>
<tr>
<td>Genealogy is usually known</td>
<td></td>
</tr>
<tr>
<td>Each culture is usually karyotyped</td>
<td></td>
</tr>
<tr>
<td>Storage is in liquid nitrogen vapor phase, -197°C</td>
<td></td>
</tr>
<tr>
<td>A duplicate collection is kept in a separate tank</td>
<td></td>
</tr>
<tr>
<td>Tanks have alarm systems</td>
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<tr>
<td>Estimated cost: $19/year/sample</td>
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</table>

What we have been collecting is virtually everything that has come by our door in the animal world. We have made use of autopsies, and sometimes very fresh animals have been available. In our autopsy room at the San Diego Zoo, all dead animals are autopsied, and from almost all of them we have collected specimens. Those that have grown well and were not somehow contaminated have been placed into the collection. It is a large collection of duplicate cultures very well characterized by species and very often by the point of origin. The genealogy of each culture is indexed in a computer program that lives in the VAX at the University of California and usually with a number in the International Species Identification Program (ISIS) at the zoo in Minneapolis, Minnesota. ISIS has a stock list of all the animals in zoos in this country and in the world, more or less. A reference point from our collection to that collection is available. This is of importance particularly when breeding animals.

Because of our interests and because of the profusion of Artiodactyla at the San Diego Zoo, our collection consists of a large number of Artiodactyla. There are lots of primates and other animals. These are invaluable for the researcher who is working in genetics or evolu-
tion of mammal species. Without our collection, research material would be totally inaccessible. One could not possibly get 10 gorilla or 20 dolphin specimens from anywhere else.

Permanent records are kept in duplicate, in books as well as backed up in a computer. We have our own in the laboratory, and it is connected to the VAX at the University. Most of these specimens are karyotyped and reasonably well defined. We have two of these large tanks, and they are automatically fed with liquid nitrogen. There is an alarm system, but unfortunately both tanks are in the same building. There is no further backup available for any of this. The collections are deep frozen in tissue culture fluid, very well indicated, and located easily. More recently we have some sperm and ova. The freezing takes place under preset conditions by means of a programmable, computerized freezing apparatus. This permits the protocol to be optimized for each species that is frozen.

In our collection are an over-abundance of Artiodactyla, primates, Perissodactyla, or their relatives, a few bats and insectivores, and other little critters which, unfortunately, are not being collected by very many people. There is, of course, the one collection of bats and rodents at Texas A&M University, but, by and large, a greater amassment of these animals is not available in any laboratory to the best of my knowledge. At the American Type Culture Collection (ATCC) the mammalian material is mostly human cell lines. We have tried to collaborate with ATCC, and I hope this will work in the future more satisfactorily. They are not really interested in storing a large number of lines from different mammals, and there is no one else at the moment that does so. T.C. Hsu at Texas once collected some lines, but most of these he has sent away, having gone into cancer research.

The current supervision of the CRES activities is by Oliver Ryder who has a Ph.D. in molecular genetics with a great interest in comparative genetics and evolution. The collection continues to be supported by the Zoological Society of San Diego, largely through its Center for Reproduction and Endangered Species. Much of the actual work going on with the collection is being funded by research grants that contribute a few dollars here and there, but that is not the major portion of it. In calculating the real costs, it is very difficult to arrive at a figure for storing a sample per species per year. We have come up with about $19 a year. That may seem very high since the sample only sits in liquid nitrogen. But this is the actual cost of freezing it down, unfreezing it, checking it out, karyotyping it, refreezing it, verifying it every now and then, and entering it into the computer.

We are continually being asked by many investigators to ship them tissues of animals that are virtually unattainable by them. This is a major problem for us, because it takes time away from our own research activities. We are not there to make money on it; in fact, our billing for shipping is very improper and not very well handled. The real problem is international shipment of animal tissues and the im-
porting of tissues from other countries. I have personally a great interest in South American animals, particularly Artiodactyla, and find it virtually impossible to bring material from South America due to USDA restrictions. Likewise there are great difficulties bringing anything in from Africa and other countries. One should abide by the legal constraints put upon us. None of us would like to bring in hoof-and-mouth disease viruses or other agents that are potentially dangerous if disseminated throughout the world. It is for this reason, in part, that I think our collection is very important. It was established from animals that were already here and were post quarantine and certified not to have hog cholera or some other dreadful disease that might raise havoc with livestock in this country.

We use the collection extensively for the interpretation of the cytogenetic evolution of animals. More importantly, we have used it for studying the ever-increasing problems of infertility that we see in zoological parks. One of my more interesting recent observations has been when we tried to characterize Soemmerring’s gazelle (Gazella soemmerringi) which comes from Somalia. In collections they have died out regularly after a few crosses. I collected specimens of Soemmerring’s gazelles and karyotyped them. There turned out to be nine different chromosomal variants in these specimens. That is, there were numerical chromosomal variants, in addition to major translocations which conferred sterility on the offspring. In fact, we have no idea what the karyotype of Soemmerring’s gazelle actually should be, because we have nine different karyotypes from animals identified by curators as Soemmerring’s gazelles which allegedly come from the Somalian highlands.

At the moment we are studying dik-diks, some of which are monotypic, monospecific actually. Dik-diks have at least four different chromosomal variants which render them subfertile or infertile. For the breeding of animals in the future it is essential that one have this information, otherwise one will never get self-sustaining populations of animals that have become extinct in the wild or are no longer obtainable.

We have a number of guest investigators and ship material out to other researchers. Moreover, recently the American Zoologic Society and the American Association for Zoological Parks and Aquariums have recognized the necessity to form species survival plans for the animals that are almost gone. These include several ‘emblem’ species, but there are many others needing attention. For them we are doing a lot of different types of work. At the moment our work is only in mammals, but we hope in the future it will be in birds as well. We are beginning, for instance, with the California condor, to fingerprint its genome.

One thing has been troubling me a lot. I have the ability only to freeze mammalian cell lines. I am not a cryobiologist; I know nothing about the ability or lack of ability to freeze avian cells. There are many
large birds like the California condor whose genome we would like to
study in the future, but I know nothing about our ability to freeze them.
The same goes for reptiles. Such expertise needs to be developed in
the future. I would hope it could occur in a collection center like that
proposed at Davis.

In any event there are many future uses for the collection. They
are challenging in the current context of better understanding the
DNA of mammals and their evolution. It is important to have available
at least the DNA in the form of replicating cells. It is better than hav-
ing nothing in the future. We would like to make DNA libraries and
have done a few, but we have not even begun to scratch the surface on
a systematic basis.

It is true that the public, by and large, looks upon the content of a
frozen zoo as something from which you can make rhinoceroses or go-
rillas after the rhinoceroses or gorillas have become extinct. They look
upon this as a wondrous machine with which to reproduce animals in
the future. Unfortunately, some of the publicists in our zoo and in gen-
eral have misled the public in this respect. It is true we are trying to
learn how to freeze, say, rhinoceros semen or semen from giraffes. But
in my own view this is not a very likely success story for the future, be-
cause there will be no more rhinoceroses or giraffes into which one can
inject the semen. It would be very difficult at the moment to collect
semen of Sumatran rhinoceroses because there are only four in cap-
tivity (one male only), and nothing is known about their biology. Most
curators in most zoos would not allow one to touch one of those ani-
imals for electro-ejaculation or other purposes. So for the future of the
systematic collection of genomes that are potentially replicating, such
as semen or fertilized eggs, the Center is not yet ready. I think the
world is not ready. At the moment we are trying to conserve primarily
DNA and cell strains so our colleagues in the future have it available.

DISCUSSION

Question: Which phase of nitrogen is used for storage, the liquid or vapor phase?

Benirschke: Actually the storage occurs above the liquid in the vapor phase. But
it is automatically fed to a level that we predetermine. The survivability of these
strains is very good indeed.
CONSERVATION OF GENETIC RESOURCES: A PROPOSAL FOR SHARING THE RESPONSIBILITY

Calvin O. Qualset

Abstract: Genetic resources include (1) populations of organisms in their native habitats and wild types maintained in zoological and botanical gardens, in seed or gamete banks, or microbial cultures; (2) plants, animals, and microorganisms that evolved through direct and indirect genetic manipulation; and (3) highly defined stocks arising as the result of genetic research. Genetic resources must be conserved indefinitely for future scientific, technological, and evolutionary advances. Those who benefit from conserved genetic resources are many and diverse. It is not always clear where the responsibility for protection of genetic resources lies; however, both governmental and nongovernmental, national and international consortia should establish criteria, develop genetic resources management plans and priorities, and arrange funding. Funding is the most crucial step. Multiple sources of funds are desired, and wide-scale public involvement is essential. Major successful efforts can be cited for parks, reserves, seedbanks, and botanical and zoological gardens. Special genetic stock collections of many species have been encouraged and developed by short-term research funding, and thereafter orphaned. A secure base-level funding system should be developed in the US as a national endowment or trust fund derived from both public and private funding sources. A portion of research funding should be assessed for genetic resources maintenance. For the US, a National Biological Resources Agency should be organized to ensure the permanent security of biological resources for future generations to use and enjoy. This concept can be extended internationally for all types of genetic resources.

Calvin O. Qualset received his B.S. in Agriculture from the University of Nebraska in 1958 and Ph.D. in Genetics from the University of California, Davis in 1964. He served on the faculty of the University of Tennessee from 1964-1967 and since then at the University of California, Davis where he is presently a Professor and Director of the California Genetic Resources Conservation Program. His research interests include the genetics, breeding, and evolution of cereal crops, especially with reference to genes conferring broad and specific adaptation in natural populations and cultivars of species of the Triticeae. He has broad interests in biological conservation and its facilitation.
There are rapid economic, social, and agricultural changes throughout the world that are impacting greatly on availability of genetic resources to meet the needs of a rapidly expanding human population. Throughout this symposium there has been emphasis placed on availability and accessibility of genetic resources for research and for development of new products, such as antibiotics or crop varieties. Genetic resources are extremely vulnerable to loss at the present time, and it was the intent of this symposium to call attention to this vulnerability and then to offer suggestions for overcoming this alarming situation. It is the intent of the following discussion to summarize some of the major points about genetic resource conservation. Underlying this discussion will be an emphasis on the commonalities that exist between the practices of conservation of specific genetic resources and the practices of conservation of biological diversity in its wild state. Finally, a coordinated action and funding plan is proposed which considers biological resources in the broadest sense as the target of conservation.

Types of genetic resources:

**...natural populations**

One class of genetic resources consists of the natural populations of wild species of organisms that have evolved, and are still evolving, over a long period of time. They can be maintained by habitat preservation. As a last resort, small samples can be saved by *ex situ* conservation in gardens, zoos, and microbial cultures, but samples of most species from the natural populations are necessarily small and do not adequately represent the genetic diversity within the species.

**...landraces**

A second type of genetic resources are the landraces of agriculturally important species of plants and animals. Landraces have been developed without serious or conscious plant or animal breeding, but they have been modified over millennia by farmers. Possibly these can be retained *in situ* in natural or native farming systems. However, Dr. Rick (p. 12) has pointed out, in certain cases, *in situ* conservation of these landraces is not very secure. Thus, *ex situ* conservation in seed banks, plantations, botanical gardens, or zoos is the primary method.

**...germplasm stocks**

A third type consists of germplasm stocks and genetic stocks. These can be roughly considered as “manipulated genetic resources.” These are products of breeding, selection, and creation of special gene combinations by induced mutation or molecular manipulation. Germplasm stocks are breeders’ lines that come from the manipulation of populations. They appear in various forms as livestock breeds, plant cultivars, or microbial strains. These are primarily retained by *ex situ* conservation. But some of them in fact, are not endangered as breeds or strains because they are so widely grown throughout the world, as is, for example, the Holstein dairy cow or the Leghorn chicken.

**...genetic stocks**

Genetic stocks are very special genetic resources that are well defined and almost always must be maintained by *ex situ* conservation methods. They are expensive to maintain. Most of the collections of
defined genetic stocks do require special study or manipulation when they are regenerated, for example, to verify their chromosomal status or to select specific mutant gene combinations.

From a utilitarian point of view, one issue is conserving genetic resources of species used for food, fiber, and energy as they may be needed in the future. From the scientific point of view, conservation permits evolution to proceed (in contrast to preservation which retains static gene combinations), so that evolution and interrelationships of species and genes can be studied. From the environmental point of view, biological conservation is a vital component in maintaining the quality of water, soil, and atmosphere. Finally, conservation is important for sustaining agricultural systems and for aesthetic qualities.

The conservation of all of these forms of genetic resources requires careful analysis in terms of numbers of species, amount of genetic variability within species, and native habitat — all of which are subject to government policy (or lack of it), individual commitment to conservation, available trained human resources, and, of course, financial resources.

Since these types of genetic resources are interrelated, the conservation of biological diversity, i.e., conservation of genetic resources, should be approached in an integrated way. Operationally, genetic resource conservation programs have the following components: The first is to develop scientific principles for genetic resource conservation. This means applying genetic and biological principles to conservation in a sensible way. The second is to establish criteria for conservation of the various types of genetic resources. For example, what are the criteria for conservation of natural populations? These must be cognizant of the specific requirements for species and its environment. The third step is to develop a workable strategy to conserve a specific group of genetic resources. The fourth step is to create an implementation plan. This is where the rub begins. The first three steps can be done on the basis of prior knowledge, research, and good sense. Implementation, however, requires interaction among people who have responsibility for the genetic resources that are objects of conservation. Institutional requirements and arrangements, including legal aspects of ownership, have to be dealt with. The availability of human, physical, and financial resources are often limiting.

Another point of emphasis is that the conservation of genetic resources of tropical forests, for example, is fundamentally no different than conservation of genetic stocks of maize or mice. The planning and implementation phases are different, but it is still the same basic problem. Both ends of that spectrum must be given proper attention. Comprehensive genetic resource conservation plans are needed in which the interrelationships of *ex situ* and *in situ* requirements for conservation are included. These plans must also establish responsibility of organizations or individuals for the conservation of the targeted genetic resources.
One of the problems is that conservation is forever. In other words, a conservation program cannot be started one year with the possibility it will be changed on the whim of the next administrator or government agency a year or so later. Continuous, permanent attention is required. It must be stable over time and must have a degree of independence from politics, both domestic and international. The latter is especially important because of the global aspects of genetic resources conservation.

What is happening now? Most of the present effort and attention is given to natural populations and habitat preservation. There are certain limitations that need to be urgently addressed by the scientific community. What information is available or needed in terms of the biosystematics and genetic diversity in natural populations? That certainly has a bearing on the strategy of conservation to be adopted. There has typically been insufficient planning. There is a time factor problem because of rapid urban development and environmental effects induced by agricultural, forestry, and industrial activities. Spontaneous decisions must be made about conservation that may or may not be correct. Currently, there are insufficient human, physical, institutional, and financial resources marshalled to deal properly with preservation of the genetic variability in natural populations and in habitat preservation.

In terms of ex situ conservation of plants and animals, there are many species represented in collections in botanical gardens, zoos, seed banks, and so forth. However, they do not really represent the range of genetic variability as it occurred in nature. Of course, many such collections were not brought together for that purpose. They were brought together as demonstration materials for teaching purposes or for research, truly representative collections exist for only a very few species.

In terms of the manipulated genetic resources, i.e., the germplasm and genetic stocks, permanent funding and organization networks are really not well established. There is essentially none for domestic animals. There is a fragmented one for microorganisms. For cultivated plants, organized conservation activities are much more developed than for other organisms. For example, there is a rather comprehensive National Plant Germplasm System in the United States, and there is a large international activity by the International Board for Plant Genetic Resources; but the primary attention is given to domesticated rather than wild species. The Center for Plant Conservation is evolving a network for ex situ conservation of rare plants.

Genetic stocks are practically left out of the general conservation issue. That is partly because they are usually developed by very specific research programs funded by specific research grants. These grants may be awarded on a competitive, peer-reviewed basis. If a proposal is not funded or a continuation grant is denied, the genetic materials cannot be maintained. Thus, the process which successfully advances
scientific knowledge is a direct contributor to the loss of genetic stocks that are needed for further advancement of science. Clearly, a mechanism is needed for long-term funding for the maintenance of collections.

The conservation of wild and domesticated genetic resources is a matter of national importance in the US, even to the extent of impacting national security for food and natural resources. At the federal level there is no single agency having responsibility for monitoring or managing genetic resources. This could be provided by a Biological Resources Conservation Agency in federal government which would have direct oversight and management responsibility for biological materials held in the public domain. It would monitor and provide financial assistance for the conservation and distribution of genetic materials held by public nonfederal organizations. This national system could be modeled after the present National Plant Germplasm System operated by the US Department of Agriculture in its Agricultural Research Service and Cooperative State Research Service. It would be broadened to include units for conservation of biological diversity of naturally occurring species, including rare and endangered ones, and additional units or systems for livestock, including fisheries, and microbial species. This unified concept for management of biological diversity in the US is attractive from the point of view of providing clear responsibility to a federal agency, somewhat analogous to the Soil Conservation Service in the USDA, for biological resource conservation.

Most important from an operational point of view is that mechanisms for providing operational funds for the Biological Resources Conservation Agency could be devised. Here it is clear that multiple funding sources are needed. Much biological conservation activity, as for example in the zoos and botanical gardens, is done with a mixture of private and public funds. This should be encouraged, with federal funds being provided for special needs. Line-item funding is required, but additional funds could be attracted to a National Endowment for Genetic Resources — a "social security" system for genetic resources. This endowment fund could be developed by direct ear-marked donations and by private-federal matching to encourage substantial donations. The endowment funds would provide operational funds for conservation programs from the interest earned and would ensure the permanence that is essential for conservation of items of importance for the national security. The US has a good, but not outstanding, record in biological conservation. A unified approach would provide a model for other nations to follow. At the same time, we could learn from other countries, Great Britain, for example, where some elements of this proposal are already functional.
DISCUSSION

Comment (from audience): Groups like the Sierra Club and other groups with political bases might be useful in creating an issue of this tie between genetic resources and our long-term national security.

Qualset: I agree. I think we have to articulate the conservation issues more broadly in a biological and scientific sense. The action of setting aside more land or waters in preservation reserves is important, but I think we must know what is the biological content in those places. We must be sure that we have a total program, one that is going to involve both in situ and ex situ conservation.

We have had many examples of industrial or urban development which impinge upon endangered species. There are examples of impacted populations of such species being moved to other, secure places. This is an operation of last resort to mitigate the impact. It is the developer who must pay for this mitigation cost and for the maintenance of the populations in the new sites forever. The developers establish a trust fund to finance this perpetual maintenance. The actual moving and maintenance is done by an organization with established expertise. For example, with funding from developers, the Rancho Santa Ana Botanical Garden in California has accepted responsibility for mitigating development impact in this way in at least one instance. This procedure could be a model for funding other aspects of conservation. We have to deal with the practicalities of the human population pressure.

Question: Could you identify the areas you feel are most under pressure in terms of loss of diversity, areas either in terms of species or in terms of actual geographic areas in California?

Qualset: For example, there are wild species of Fragaria related to the commercial strawberry in California, and many of their populations have been threatened and destroyed by development in coastal areas. There are other plants that are endangered by development aspects. An example is the native annual meadowfoam (Limnanthes) which grows near vernal pools. This plant produces seed which contains industrially useful oils. There is also the matter of overgrazing that threatens certain species.

Question: Where is the major concentration of funding in California? Is it for conservation of wild species or for economic species?

Qualset: I cannot give you a precise answer on that. We are doing some survey work of what portion of public funds and grants for research are spent on actual genetic resource conservation practices, but we do not have answers yet.
Most of the deliberate conservation is directed toward rare and endangered species. There are government agencies and private voluntary organizations working directly on that problem. We have a state environmental fund which is developed by charging a fee for car license plates with personalized messages. Those funds are used almost entirely for conservation of rare and endangered species, including land acquisition. What we are seeking is a way to raise the more general consciousness of conservation. Certainly, in California and other states, rare and endangered species raise the general awareness of the conservation issue. For example, the impact of agricultural practices on biological diversity is now surfacing. The use of pesticides that could harm an endangered species is being curtailed. In the last few years, there have been model programs set up in counties across the country. Merced County in California is an example. First, the species there were inventoried and the number of rare and endangered species of insects, plants, or animals, was determined. Then a list of pesticides that might affect these species was drawn up. The idea is to eliminate the use of those pesticides. At this point, the farmers are impacted. A lot of questions are raised. Was the biological survey adequate? How precisely known is the distribution of these taxa? Was the toxicological data adequate? If there is an insect endangered there, is its habitat so well defined that pesticides could be judiciously used? These are very troublesome issues for agriculture. They are also troublesome from the endangered species point of view. More effort on research and on biological surveys is required. There are many conservation issues that impact on agricultural species. It is not only endangered species that are impacted by agricultural activities.

Comment: Endangered species will always be with us. You have suggested that we have perhaps a fairly adequate system at the present time for agricultural plant species. In general, it has been tough to implement new projects with limited resources. I like very much your idea of an endowment. But how an endowment is set up, of course, becomes somewhat of a problem. It seems that with the success of the biotechnology industry, there could be a source of money there, if for no other reason than as a tax write-off.

Qualset: The genes are free, basically. We have wanted the resources to be distributed freely so people could have access to them and not be required to provide a certain amount of money to use them.

Comment: I am not suggesting a fee for samples, though, that may be what you have to come to. But with respect to plants, Pioneer Hi-Bred International made a monetary commitment to conservation over periods of time. If that could be put into an endowment instead of something that is spent next year, next year, and next year, then your “forever” becomes realistic.
Qualset: That is a good point. What I meant by the genes being free is that paying for them has not been a part of any business plan of a biotech company or any other company. The seed companies know that the genetic resources are available to them. They budget for other issues, such as getting enough money to operate labs, but they do not have to allocate any part of their money to get their initial stocks to work with. We are going to pursue this endowment idea with the University of California Tomato Genetics Stock Center. This Center was reviewed and a report* was prepared showing how valuable the Genetic Stocks Center is, what it contributes, and how there has been a considerable industry interest in it. We are hoping that between the public and private sectors, an endowment fund could be established. If it can be done for tomatoes, it will be a good model for other species.

Comment: If genetic resources literally represent financially immeasurable value in terms of the world's future, it seems that this idea of the endowment could be modeled after The National Endowment for the Arts created in the 1930s in America. The way Congressional and Presidential powers were brought to bear to create it would be instructive to study. There must be a grassroots understanding of why these things really have an actual future value.

Comment (Allard): When one considers maintenance of diversity at the level of the genotype, it becomes quickly obvious that any ex situ facility would easily be swamped by the numbers of genotypes of just one species. I would suggest making alleles the criterion of selection rather than these incredible numbers of genotypes. The question becomes whether one can reconstitute populations of genotypes from stocks maintaining alleles. As a matter of fact, in the particular case of barley, it turns out that it is very easy to reconstitute the population. At least one can obtain the alleles that are favorable in a local environment. It turns out that worldwide there are only about half a dozen major environments for barley to be concerned about. The procedure would be to make up an arbitrary population of individuals containing identified alleles. That is, one would put them together and grow them in large numbers. It has to be in large numbers, but there would be only one population. Within 15 or 20 generations of growing that population in a given environment, the desired alleles are expected to be in fairly high frequency.

Qualset: This is an excellent point which illustrates how a genetic resource conservation strategy can be developed for a crop plant or species. Clearly, Noah's ark would be full very quickly if everything were to be brought into ex situ conservation status.