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

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

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

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

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

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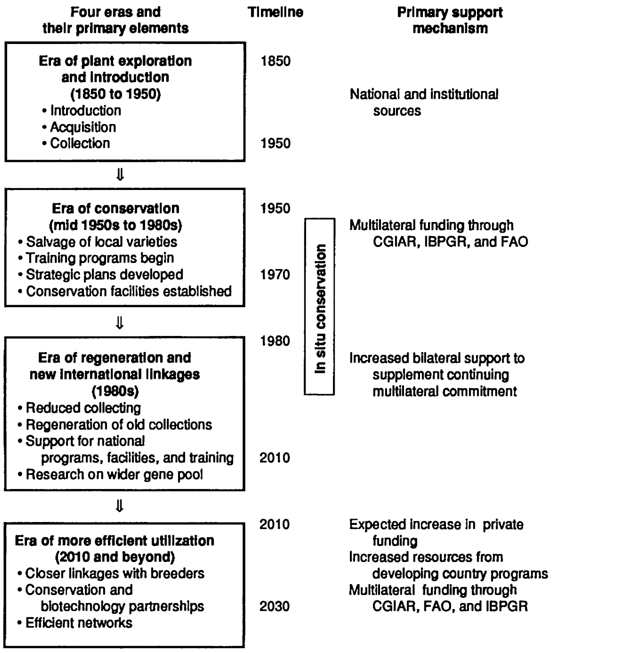

Fig. 1. Four eras of ex situ genetic resource conservation and use, with timeline of conservation events.

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

Development Assistance for in Situ and ex Situ Conservation

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

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

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

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

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

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

Four Eras of ex Situ Activities and Development Priorities

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

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

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

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

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

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

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

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

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

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

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

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

Recommendations for Action

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Exploring Available Funding Options

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

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

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

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

Donor Concern Regarding ex Situ Conservation Options

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

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

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

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Research Articles

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

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

The principal biological role of acetylcholinesterase (AChE, acetylcholine hydrolase, E.C. 3.1.1.7) is termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (ACh) (*1*).

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

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

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