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Ecology

Saving the "library of life"

(biodiversity/population biology/cell bank/frozen zoo)

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ABSTRACT **A broad program of freezing species in threatened ecospheres could preserve biodiversity for eventual use by future generations. Sampling without studying can lower costs dramatically. Local labor can do most of the gathering. Plausible costs of collecting and cryogenically suspending the tropical rain forest species, at a sampling fraction of 10^{-6}, are about 2 billion dollars for a full century. Much more information than species DNA will be saved, allowing future biotechnology to derive high information content and perhaps even resurrect then-extinct species. Parallel programs of *in situ* and other *ex situ* preservation are essential to allow later expression of frozen genomes in members of the same genus. This is a broad proposal that should be debated throughout the entire scientific community.**

We have only begun the elementary taxonomic description of the world biota. While about 1.4 million species have been given scientific names, estimates of the total number of species range up to roughly 30 million or higher (1, 2). Systematists may very well not know the species diversity of the world flora and fauna to the nearest order of magnitude.

Time is running out in which we can even catalog our living wealth. Though the overall extinction rate from fossil evidence is of the order of 10^{-7} species per species year (3), a very conservative estimate of the current extinction rate gives roughly 5000 species lost annually (4), with some values far higher (5, 6).

We are accelerating toward a calamity unparalleled in planetary history. The best-known cause of present-day species extinction is the cutting of tropical forests, which have lost about 55% of their original cover and are shrinking at the rate of 1.8% per year (5). Worse, the rate seems doomed to increase, since its ultimate cause is human activity, and human numbers and expectations grow apace. To improve the lot of a swelling human tropical population would require at least a 5-fold increase in economic activity there, bringing a crushing load on the already strained biosphere (6).

Other biological zones such as coral reefs and oceanic islands also dwindle at alarming rates. Because of the latitudinal species diversity gradient, losses are most severe in precisely the tropical continents where our own numbers swell so alarmingly (7).

Everywhere there are calls for a halt to tropical deforestation, but most voices seem tinged with despair. Ehrlich and Wilson (6) suggest that we could lose a quarter of *all* species in half a century, with incalculable effects on our biosphere. We now co-opt about 40% of net photosynthetic productivity worldwide, favoring monocultural crops, which must greatly affect genetic diversity. Given the blunt economic and cultural forces at work, even slowing the rate of destruction seems doubtful in the immediate future.

This dire moment demands radical thinking. In the spirit of a thought experiment, I discuss here a proposal that links the *in situ* preservation community, which emphasizes protected wild areas, and the *ex situ* conservationists such as zoos, botanical gardens, etc. For *in situ* measures there are economic, environmental, and aesthetic arguments. To preserve the genome of many species, however, *ex situ* methods may suffice. Considering this possibility serves to separate the kinds of arguments we make for conservation methods, including concepts of our moral debt to posterity. In the spirit of sharpening debate by considering plausible scenarios, we can test our ideas.

Salvage by Sampling

Our situation resembles a browser in the ancient library at Alexandria who suddenly notes that the trove he had begun inspecting has caught fire. Already a wing has burned, and the mobs outside seem certain to block any fire-fighting crews. What to do? There is no time to patrol the aisles, discerningly plucking forth a treatise of Aristotle or deciding whether to leave behind Alexander the Great's laundry list. Instead, a better strategy is to run through the remaining library, tossing texts into a basket at random, sampling each section to give broad coverage. Perhaps it would be wise to take smaller texts, in order to carry more, and then flee into an unknown future.

While efforts to contain and control our accelerating biodiversity disaster are admirable and should be strengthened, it may well be time to consider a similarly desperate method of salvage. I propose that the biological community ponder a systematic sampling of threatened natural habitats, with long-term storage by freezing. This would more nearly resemble an emergency salvaging operation than an inventory, for there would be minimal attention paid to studying the sample. The total sample mass might be reduced by judiciously trimming oft-repeated species of the prolific ants and beetles. (Duplication in sampling makes for good statistics, though, helps comparative anatomy, and may aid those for whom more successful species are more interesting.) The essential aim is to save what we can for future generations, relying on their better biological technology to extract the maximum benefit.

Sampling of tropical trees by insecticidal fogs and active searching of the canopy is common. Teams trained to simply collect, without analyzing, require minimal labor by research biologists. Freezing at the site can be done with ordinary ice or dry ice; liquid nitrogen suspension can occur only at the long-term repository. Extensive work by taxonomists enters only when samples are studied and classified. Here lies our current bottleneck. There are far too few taxonomists to tally the world's species within our generation (6), let alone analyze them.

We sidestep this problem if our primary aim is to pass on to later generations the essentials of our immense biodiversity. Even information about the existence of a species is useful, because without a sample, in the future one cannot be

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sure whether a given variation did not exist at all or simply became extinct without being observed. Thus extensive taxonomic expertise would defeat the project. Detailing allelic variations within a population or other variations (such as conspecific, interpopulation, congeneric, ecosystem, etc.) probably will not be worth the trouble.

It seems likely that captive breeding programs, parks, microhabitats, and zoos can preserve only a tiny fraction of the threatened species. (Here I shall use the term "preservation" to mean keeping alive representatives of at least each genus—*in situ, in vivo* protection in reserves—and argue that this is essential to eventually studying and potentially resurrecting frozen species.)

To save the biosphere's genome heritage demands going beyond existing piecemeal strategies of seed banks, of germ plasm and tissue culture collections, and of cryopreservation of gametes, zygotes, and embryos; these programs mostly concentrate on saving traditional domesticated varieties (8). Our goal is a complete sample of all threatened species.

Cryoprotection

Much more than data about existence can be carried forward by simply preserving a wide sample at low temperatures. Banking cells by drying them with silica gels, for example, is useful for short times, but at room temperatures thermal damage to DNA will accumulate over the decades. We know that seeds can germinate after lengthy freezing and that microbes can sustain cryogenic temperatures. Simple cells such as sperm and ova survive liquid nitrogen preservation and function after warming. Generally, organs with large surface-to-volume ratios preserve well, such as skin and intestines.

Of course, more complex systems suffer great freezing damage, though research proceeds into minimizing this. Several kinds of damage occur, and little is known about methods of reversing such injury. Biochemical and biophysical freezing injury arises from shrinking cell volume as freezing proceeds. Plants display extrusion of pure lipid species from the plasma membrane, as cells contract during freezing (9, 10). Such lipids do not spontaneously return to the plane of the membrane during volume expansion on thawing, so that restoration of approximate isotonic volume near the melting point causes cellular lysis due to inadequate membrane surface area. While osmotic injury can be reversed (11), there is loss of membrane proteins (12). Reorganization of membrane bilayer structure into cylindrical lipid tubes may be reversible with warming (13–15). Structure of the cytoplasm may break down into blobs of proteinaceous matter (16, 17). Major fracturing of cells, axons, dendrites, capillaries, and other elements causes extensive damage at temperatures below the glass point (18), suggesting that this be avoided. For some purposes, then, immersion in liquid nitrogen may be unacceptable.

The problem of recovering cells from frozen samples is complex, but even low survival rates of one cell in a million are irrelevant if the survivor cells can produce descendants. However, our minimum aim can be to simply retain DNA, the least we should expect from a sample—though, of course, suspending whole creatures retains far more information. For this, liquid nitrogen is suitable for long-term storage (−196°C), especially since it is by far the cheapest method. At 25 cents per liter, liquid nitrogen is the lowest priced commercial fluid, excepting water and crude oil. It allows suspension in large, easily tended vaults, simply by topping off the amount lost. Only a wholesale breakdown of industry can plausibly destroy the samples; no mere power failure will do. Redundant storage at different sites avoids even this.

Further, while neither liquid nitrogen nor freeze-drying damages DNA, freeze-drying does cause far more injury to structural and taxonomic characteristics. For the broad program envisioned here, which should also include tiny samples of ocean water with its teeming viruses and bacteria, plainly liquid nitrogen is essential. This is also true for saving whole creatures, since we also gain their parasites, bacteria, and viruses, which are better preserved cryogenically.

A crucial point is that we need not rely on present technology for the retrieval. Progress in biological recovery can open unsuspected pathways.

Recent advances underline this expectation. Techniques such as the polymerase chain reaction can amplify rare segments of DNA over a million-fold (19). Such methods have enabled resourceful biologists to recover specific segments from such seemingly unlikely sources as a 120-year-old museum specimen, which yielded mitochondrial DNA of a guagga, an extinct beast that looks like a cross between a horse and a zebra (20). A 5000-year-old Egyptian mummy has given up its genetic secrets (21). Amplifiable DNA in old bone is beginning to open study of the bulk of surviving organic matter from the deep past (22). The current record for bringing the past alive in the genetic sense is DNA extracted from a *fossilized* magnolia leaf between 17 and 20 million years old (23). This feat defied the prediction from *in vitro* estimates of spontaneous hydrolysis rates, which held that DNA could not survive intact beyond about 10,000 years (24).

We should recognize that future biological technology will probably greatly surpass ours, perhaps exceeding even what we can plausibly imagine. Our attitude should resemble that of archeology, in which a fraction of a site is deliberately not excavated, assuming that future archaeologists will be able to learn more from it than we can.

Preserve the Genus, Freeze the Species

We need a combined strategy to salvage biodiversity out of catastrophe. The best approach may be two-pronged:

(*i*) preserving alive some fraction of each ecosystem type ("biome"), its population represented intact at the genus level, and

(*ii*) freezing as many species related to the preserved system as possible.

At a minimum, this will allow future biologists to extract DNA from frozen samples and study the exact genetic source of biodiversity. Genes of interest could be expressed in living examples of the same genus, by systematic replacement of elements of the genetic code with information from the frozen DNA. Obviously, the preserved genus is essential.

These techniques would open broad attacks on the problem of inbred species. A ravaged environment can constrict the genetic diversity of individual species. Reintroducing diverse traits from frozen tissue samples could help such a species blossom anew, increasing its resistance to disease and the random shocks of life.

Beyond this minimum—the DNA itself—future biologists will probably find great use for recovered cells in reexpressing a frozen genome. Cell use for mollusks, trees, insects, etc. is a cloudy, complicated issue. For mammals, uterine walls, elements of the sexual reproductive apparatus, etc. should prove essential, since placentation and the highly variable physiology of different taxa are crucial. It seems highly unlikely that one can make appropriate placental and endometrial choices in the many steps from genome to newborn, merely from reading DNA.

As saviors of the "Library of Life," we are at best marginally literate, hoping that our children will be better readers and wiser ones. Many biotechnological feats will probably emerge within a few decades—many ways, let us say, of reading and using the same genetic "texts." But no advanced "reader" and "editor" can work upon texts we have lost.

This holds out the hope of selectively reintroducing biodiversity in the future, to gradually recover lost ecosystems. Individual species can be resurrected from very small numbers of survivors, as the nearly extinct California condor and black-footed ferret have been.

Fidelity in reproducing a genome may not be perfect, of course. Many practical problems arise (placenta environment, chemistry, etc.), which complicate expression of a genotype. In any case, future generations may well wish to edit and shape genetically those species within an ecosystem as they repair it, for purposes we cannot anticipate.

Loss of nearly all of an ecosystem would require a huge regrowth program, for which the Library of Life would prove essential. Suppose, though, that we manage to save a large fraction of a system. Then the species library will provide a genetic "snapshot" of biodiversity at a given time and place, which evolutionary biologists can compare with the system as it has evolved much later—for example, through perhaps thousands of insect generations. This would be a new form of a research tool.

Already a crash program to collect permanent cell lines, DNA, or both from vanishing human populations has excited attention (25). This program maintains cell lines by continuous culture, a costly method that invites random mutation. Such records may allow a deeper understanding of our own origins and predispositions (26), but banking frozen tissues of endangered species is the only way to ensure that any genetic disease diagnosed in the future in small, closed populations (the "founder effect") can be mapped and managed (27). Ehrlich (28) suggested creation of "artificial fossils" in such fashion. The "frozen zoo" of San Diego, begun with this in mind (29), has immersed 2400 mammal fibroblast cell cultures and 145 tissue pieces in liquid nitrogen—about 300 species in all. Cryonic mouse embryo banking for genetic studies is now routine (30).

The far larger prospect of eventually reading and using a Library of Life is difficult for us to imagine or anticipate, at the early stages of a revolution in biological technology. Our situation may resemble the Wright brothers if they had tried to envision a moon landing within three generations.

Can We Afford It?

This sweeping proposal avoids the problem of deciding which species are of probable use to us or are crucial to biodiversity. By sampling everything we can, we avoid some pitfalls of our present ignorance. Too often, preservation efforts focus on "charismatic vertebrates," neglecting the great bulk of diversity (8).

Many conservationists may be reluctant to support a cryopreservation campaign, because they fear it will sample too sparsely. This assumes that present taxonomic methods and costs are necessary. But an important feature of this proposal is that the samples need not be studied as they are taken. This avoids the scarcity of taxonomists, speeding field work and lowering costs. Plausibly, much of the gathering can be done with semi-skilled labor.

This suggests immediately that the bulk of the funding come from "debt swap" between tropical and temperate nations, as has been used to "buy" rain forests and set them aside from cutting (8). Further, this will create a local work force that profits from controlled, legal forest work, rather than from cutting it.

As a very rough estimate, consider a sampling program that collects all life forms from a stand of a hundred trees, but not the trees themselves, for each hundred square kilometers of tropical rain forest—i.e., a sampling fraction in the range of 10^{-6}. If this costs on average 10 thousand dollars per stand (probably an overestimate), then a million square kilometers yields 10^4 stand samples costing 100 million dollars. For all the world's tropical rain forests, covering about 9 million square kilometers, the cost is close to a billion dollars.

To suspend such samples requires replacing boiled-off liquid nitrogen. Suppose we collect 10 kg from each tree, or about 10^7 kg for a million square kilometer area. Current nitrogen loss costs of a mass M presently obey a scaling law,

$$\text{cost/year} = \$400/\text{year}\ (M/100\ \text{kg})^{2/3},$$

since nitrogen loss scales with surface area. To suspend the stand-samples for all the tropical forests then demands 3.7 million dollars per year, or 0.37 billion dollars for a century. A similar argument suggests that building the repository, curatorial labor, etc. will probably require comparable costs, especially if the samples are to be readily available to researchers, with detailed labeling. Thus an estimate of perhaps a billion dollars for a century's storage seems plausible. Added to the collecting cost, we need in sum about two billion dollars. Current outstanding debt by tropical nations well exceeds a hundred billion dollars.

Of course, this does not touch upon side costs in training biologists, transport, perhaps doing some taxon discrimination, etc. Certainly the effort compares in cost with the Human Genome Project. The task is monumental; so is the potential benefit.

Traditional economics cannot deal with transactions carried out between generations. As Harold Morowitz (31) has remarked, the deep answer to "How much is a species worth?" is "What kind of world do you want to live in?"

Counterarguments

This drastic proposal does not address many legitimate reasons for preserving ecospheres intact, and it should not be seen as opposing them. Indeed, only by preserving *in vivo* a wide cross section of biota can we plausibly use much of the genetic library frozen *in vitro*.

An obvious possibility is that preservation of habitat may compete politically with a sampling and freezing program. There is no intrinsic reason why this needs to be so. They are not logically part of a zero-sum game because they yield different benefits over different time scales. Of course we would all prefer a world that preserves everything. But the emotional appeal of preservation should not be used to disguise the simple fact that we are losing the battle or to argue against a prudent suspension strategy.

Further, sampling is far less expensive than preservation—which is why it is more likely to succeed over the long run. Even competition for debt swap funds will not necessarily be of the same economic kind. Conservationists seek to buy land and set up reserves, putting funds into the hands of (often wealthy) landowners. A freezing program will more strongly spur local, largely unskilled employment, affecting a different economic faction.

Further, a freezing agenda could call forth local programs to train people and build institutions for the study, wise management, and preservation of organisms. Getting developing countries involved with their own biodiversity problems is crucial to any long-term strategy.

Sampling and freezing have little aesthetic appeal. To some they will smack of fatalism; it may be merely realism. Also, freezing species does not offer the immediate benefits that preservation yields. (Samples would probably be taken only from areas not already highly damaged.) More concretely, this proposal will not hasten benefits from new foods, medicines, or industrial goods. It will not alter the essential services an ecosphere provides to maintenance of the biosphere. We should make very clear that this task is explicitly designed to benefit humanity as a whole, once this age of rampant species extinction is over.

Some will see in this idea a slippery slope: to undertake salvaging operations weakens arguments for biodiversity preservation. To avoid this, the two *parallel* programs of preservation and freezing must be kept clear. In this sense, the analogy to the library at Alexandria is false—for us, there is no true conflict between fighting the fire and salvaging texts. Further, in the real world, funds for conservation of DNA today do not come directly from *in situ* programs. If the Topeka Zoo budget is cut, the city does not transfer funds to Zaire to save gorillas.

Indeed, one can make the opposite argument—that the spectacle of the scientific community starting a sampling program will powerfully illuminate the calamity we face, alerting the world and stimulating other actions. Beginning with local volunteer labor and contributions—say, with the Sierra Club sampling the redwood habitat—could generate grass-roots momentum to overcome the familiar government inertia. In larger campaigns, by requiring that samplers accompany all legal logging operations, we can help develop a local constituency for controlled harvesting.

Perhaps the most difficult argument to counter is basically an unspoken attitude. As scientists, we are trained to be careful, scrupulous of overstating our results, wary of speculation—yet these militate against the talents needed to contemplate and prepare for a future that can be qualitatively different from our concrete present. Paradoxically, we scientists labor to bring about this changed future. Now is the time to bank on the expectation that we will probably succeed.

Leading figures in biodiversity argue that a large-scale species dieback seems inevitable, leading to a blighted world, which will eventually learn the price of such folly (6, 8, 30). The political impact of such a disaster will be immense. Politics comes and goes, but extinction is forever. We may be judged harshly by our grandchildren, our era labeled the "Great Dying" or the "Age of Appetite."

A future generation could well reach out for means to recover their lost biological heritage. If scientific progress has followed the paths many envision today, they will have the means to perform seeming miracles. They will have developed ethical and social mechanisms we cannot guess, but we can prepare now the broad outlines of a recovery strategy, simply by banking biological information.

Such measures should be debated, not merely by biologists, but by the entire scientific community and beyond, because all our children will be affected. These are the crucial years for us to act, as the Library of Life burns furiously around us, throughout the world.

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