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DNA Net Earth

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Human activity has dramatically accelerated the extinction of species. Man-made habitat alteration has been the leading cause, in combination with direct exploitation. Now climate change threatens to increase extinction rates even more. Adaptation to climate change requires integration of climate impacts in planning and action for biodiversity conservation, and the overall task requires funding and action similar to that discussed—but not yet delivered—under the Framework Convention on Climate Change (FCCC) or the Convention on Biological Diversity (CBD).[1] Preservation in the wild—*in situ*—is the top priority, but it is clear that many more species will disappear and we will lose access to the genetic information they contain unless their DNA is also kept *ex situ*—in captivity, cultivation, or preserved storage.

A global network of facilities should be organized to preserve DNA for every known species and for new species as they are described. This "DNA Net Earth" will be a safety net for biodiversity that can provide genetic libraries for research and commerce, be used to recover species that are endangered, and offer the potential to selectively restore species that have gone extinct. Only a small fraction of the 1.9 million known species are currently maintained as living organisms in cultivation or captivity, or maintained frozen as viable seeds or cells. Just a fraction more species have DNA in dead cells or in an extracted form that are held in long-term frozen storage. Progress towards DNA Net Earth is limited by a lack of shared priorities. Three steps can provide a way forward: developing a website to track progress on preservation whose key information is managed directly by contributing facilities; establishing new incentives and mandates for contributing specimens, including grant, publication and permit requirements; and engaging the public in collection.

Background

Life has been present on Earth for over one-quarter of the time the universe has existed and over four-fifths of the age of Earth. The universe formed 13.7 billion years ago, and the sun and Earth formed at about 4.5 billion years. Life emerged on Earth 3.8 billion years ago, and production of atmospheric oxygen through photosynthesis began at about 3 billion years. Simple muticellular life appeared 1 billion years ago, and by 550 million years the known larger groups of more complicated animals surfaced, including jellyfish, arthropods, and fish with skulls and skeletons. About 400 million years ago, fish with pectoral limbs shuffled onto land and the first sizable plants cropped up. Plants with seeds and flowers and animals with legs and wings followed. The dinosaurs came and went (except the birds) and mammals appeared and thrived. Bipedal human-like mammals turned up several million years ago in Africa. Most *homo sapiens* (humans) now living came from a small group that slipped out of Africa between 50,000 and 100,000 years ago and spread around the globe. We know species best through familiar examples of wildlife like the African elephant, bald eagle, king cobra, bull frog, rainbow trout, giant clam, white oak, giant sequoia and saguaro cactus; and through familiar domesticated species like the dog, cat, horse, cow, pig, chicken, rice, potato, wheat and corn.

Approximately 1.9 million species have been described since 1758 when Linnaeus' Systema Naturae (the definitive 10th edition) was published with its binomial nomenclature. Significantly more are thought to exist. A 2011 paper in PLoS Biology estimates that 8.7 million ± 1.3 million species exist,[2] but some think this is an underestimate, particularly in light of estimates that 1.5 million fungi may yet to be described[3] and diverse projections that estimate up to 1 billion species of bacteria.[4] The described species fall into the categories presented in Table 1.[5]

Table 1

Vertebrate Animals	62,305
Mammals	5,490
Birds	9,998
Reptiles	9,084
Amphibians	6,433
Fishes	31,300

Invertebrate Animals	1,305,205
Insects	1,000,000
Spiders and scorpions	102,248
Mollusks	85,000
Crustaceans	47,000
Corals	2,175
Other Invertebrates	68,827

Plants	321,212
Flowering Plants	281,821
Conifers	1,021
Ferns and horsetails	12,000
Mosses	16,236
Red and Green Algae	10,134

Other	212,556
Lichens	17,000
Mushrooms	31,496
Other fungi	42,504
Brown algae	3,067
Protists[6]	108,932
Bacteria	9,557

Total Species	1,901,323

The collective importance of species to humans is so obvious as to be trivial. We would not have oxygen to breath without photosynthesizing species (including bacteria, which made the first oxygen on earth) and essentially all our food is from species. Neither of these values will be available in any significant scale from nonliving sources in the foreseeable future, and we would not survive without them. Species also are a mainstay for clothes and other coverings, structural building materials, medicines and fuel (albeit mostly fossil fuels from the residue of ancient life). We value species as domestic companions (dogs, cats) and as natural experiences. Some value species existence as an ethical proposition.

A closer question is whether every species is very important: Many species have emerged and gone extinct throughout life's history. Many species are closely related to others in a larger group with very similar attributes. Most species have no demonstrated specific value for humans. Furthermore, species definition remains a moving target despite the passage of 254 years since Linnaeus' system was published.

There are responses: Moving targets or not, species are distinct exemplars of nature's appearance, and if they are redefined by evolutionists they still will be what they are. The Great Blue Heron won't change its feathers if it is classified into a new order or regarded as a dinosaur. Furthermore, most would acknowledge that the portfolio of units that we call species is a good representation of life's diversity.

Species have indeed come and gone over geologic time without man's help, but we have accelerated the extinction rate. A 2001 Global Biodiversity Assessment[7] put the extinction rate over the past 400 years at 100 to 200 times greater than the "natural" background rate before modern civilization. This range fits an estimate for mammal and bird species whose numbers and extinctions over the past 400 years are reasonably well documented and can be measured against an estimated mean prehistoric lifespan of 4 million years. Whatever the exact number, it is clear that we have accelerated species extinctions, and it is also clear that climate change will increase the rate of loss.[8]

The value of some species is evident. If maize species were trending to extinction in the wild, we would still cultivate them to maintain genetic assets for agricultural corn. But is *every* species so very important, other than to itself? Many beetle, ant and fungus species are indistinguishable from each other on all but the closest inspection. Are there not likely to be some species whose preservation or disappearance would leave no mark on us? Yes. The problem, however, is that we know too little about them to choose, except for the few—mostly in agriculture—where we have established knowledge and find value. A sequence of DNA may set one species apart from an otherwise apparent twin, and that sequence may be valuable to man or in the behavior and ecology it programs the species to pursue. The only confident course is to include all species in our conservation objectives and then be realistic about what can be achieved. For conservation in the wild (*in situ*), there will be winners and losers. But we can be more ambitious with manmade (*ex situ)* genetic preservation; we can plan to preserve DNA for all known species[9] and for new species as they are described. The 1.9 million known species is not that huge a number, and published descriptions of new species require voucher specimens, which can provide tissue for storage.

Progress To Date

Many institutions are currently engaged in species genome preservation. Particularly substantial efforts have been made by agencies and consortia focused on agriculture and food security such as the U.N. Food and Agriculture Organization (FAO) and the Consultative Group on International Agricultural Research (CGIAR). Botanical gardens, zoos and aquariums maintain living collections that are generally available for public viewing. Zoos and aquariums have become particularly sophisticated at communicating their holdings to each other, driven by the need to facilitate breeding exchanges, and have revamped the International Species Information System (ISIS) so that it can be kept up to date more easily. ISIS currently includes a shared software system and

information on over 10,000 species and some 374,000 live animals located in over 800 zoos and aquariums, although the specifics are viewable online only by members and email inquiries are required for others.[10] Botanical gardens have made parallel efforts with positive results for individual gardens, such as Kew in the United Kingdom and the Missouri Botanical Garden in the United States, but without an international system matching ISIS. However, the taxonomic scope of living organisms and frozen viable seed collections for plants is well ahead of living and frozen viable collections for animals, which are extensive but limited mostly to deep collections for a few domesticated species and broad coverage only for vertebrates.

Frozen collections, central to progress on DNA Net Earth, are typically kept in liquid nitrogen or electrical coolers at temperatures expected to prevent DNA degradation.[11] Facilities for seeds include the Millennium Seed Initiative in England, which holds seeds of about 30,000 species and aims to cover 25 percent of all plant species by 2020. The arctic Svalbard Global Seed Vault in Norway houses duplicates of viable seeds kept in storage elsewhere. The U.S. National Center for Plant Genetic Resources Preservation maintains a large seed bank in Fort Collins, Colorado, emphasizing cultivated species.

For animals, facilities keeping frozen viable cells include the repositories of the National Animal Germplasm Program in the U.S., which hold some 732,868 samples covering 33 taxa (mostly species) represented in agriculture. Institutions focused on frozen animal collections for biodiversity generally have more species but fewer samples. The San Diego Frozen Zoo® reports holdings of 20,000 viable cell cultures representing 1,000 vertebrate species. Other facilities report frozen tissue holdings whose cells are typically dead but are expected to contain intact DNA. Among these, the South Australia Museum holds some 110,000 samples. The Academy of Natural Sciences in Philadelphia has about 13,000 samples representing 4,000 bird species. The American Museum of Natural History has 70,000 samples including a collection from U.S. National Parks. The Ocean Genome Legacy in Massachusetts holds 22,000 frozen samples representing 4,000 marine species, and it has extracted and is preserving DNA from 75 percent of these.

The National Museum of Natural History (NMNH) of the Smithsonian Institution has recently taken significant steps, acquiring 20 liquid nitrogen and 58 electrical coolers with storage capacity for over 4.2 million samples, making it the largest existing museum-based frozen storage facility. The facility's current priority is archiving existing Smithsonian frozen biological specimens, typically not viable cells, but the facility plans to accession samples donated from others in the future. In conjunction with this investment, NMNH has launched an internal "Global Genome Initiative" (GGI), whose vision is "preserving the genomic diversity of life on Earth" and whose goals include to "cryo-preserve 50 percent of the diversity of life in the next five years", which the NMNH interprets to mean freezing tissue from 50 percent of known genera by 2018.

Viable frozen microorganism cultures, including bacteria, protists and fungi (as well as viruses) are maintained in an array of commercial and nonprofit facilities that provide human and nonhuman cell lines for research and development. These offer huge capacity. The American Type Culture Collection (ATCC) in the United States, for example, keeps collections for others and itself in 200 freezers, including vapor-phase liquid nitrogen freezers, mechanical freezers and cold rooms maintained at -4 to -8 degrees Celsius. In addition to human cell lines, the ATCC collections include more than 18,000 strains of bacteria, 2,000 different types of animal viruses and 1,000 plant viruses. The ATCC also maintains collections of protists, yeast and fungi, with over 49,000 yeast and

other fungi strains and 2,000 strains of protists, including a collection of bacteria, fungi, and protozoans found in U.S. national parks. FisherBioservices in the United States maintains 15.5 million frozen specimens in liquid nitrogen and electrical freezers at one location in Frederick, Maryland.[12]

In general, public information for frozen collections is spotty. Most repositories do not report the number of samples they hold or the number of species represented. Information for some facilities is summarized in Tables 2 and 3 below.

Table 2: Selected Frozen Seed and Plant Tissue Collections

Institution	Country	Method	Samples	Species	Capacity
Millennium Seed Initiative Kew	UK	Refrig	50,369	30,855	
National Seed Storage Lab	USA	Refrig	384,876	6,968	1,500,000
Nikolai I. Vavilov Institute	RUS			2,500	
Plant Gene Resources Canada	CA	LN_2, Refrig	110,000		
RBG Kew	UK	Refrig	40,000		
Svalbard Seed Vault	NOR	Refrig	747,141	4,289	4,500,000

Table 3: Selected Frozen Animal Tissue Collections

Institution	Country	Method	Samples	Species	Capacity
Academy of Natural Sciences	USA	Refrig	13,000	4,000	
American Museum Natural History	USA	LN_2	70,000		1,000,000
Australian Museum	AUS	Refrig	60,000		
Burke Museum	USA		40,000		
Cincinnati Museum Center	USA		3,500		
Field Museum	USA			2,600	
Florida Museum of Natural History	USA		30,000		
Louisiana State University	USA	Refrig	30,000		
Museum of Vertebrate Zoology	USA		50,000		
Natural History Museum of L.A.	USA		3,300		
Ocean Genome Legacy	USA	LN_2, Refrig	22,000	4,000	
Oregon State University	USA		20,000		
Queensland Museum	AUS	Refrig	2,500		70,000
San Diego Zoo	USA	LN_2	20,000	1,000	200,000
Smithsonian Institution	USA	LN_2, Refrig			4,200,000
South Australia Museum	AUS		110,000		
University of Alaska Museum	USA		85,000	1,000	
University of Texas - Austin	USA		30,000		

There is currently no clear game plan or widely shared priority for preserving the DNA of all species.

There are also coalitions. The "Frozen Ark" coalition based in the United Kingdom promotes freezing tissue for endangered species. The DNA Bank Initiative, based in Germany, has a growing international membership of facilities that store DNA and it is taking a leadership role in data management issues. The NMNH recently took steps to form the "Global Genome Biodiversity Network" (GGBN), which describes its mission as to "foster collaborations among repositories of molecular biodiversity in order to ensure quality standards, improve best practices, secure interoperability and harmonize exchange of material in accordance with national and international legislation and conventions." About 30 organizations, including the DNA Bank Initiative, have joined the GGBN, and there are meetings and discussions underway.

So far, however, most species are not represented in living collections or in frozen collections of viable or dead cells. Insects, for example, are poorly represented even though they make up half of known species. The reasons for this require examining the challenges and key questions.

Challenges to Progress

There is currently no clear game plan or widely shared priority for preserving the DNA of all species. This may reflect a perception that immediate economic and social returns are limited. The most important crops and livestock species are reasonably well covered in cultivation, captivity, seed banks and cell cultures. Other species with known high value, such as those with uses in medicines, are being investigated by public and private enterprises since they are considered promising. However many species may have undiscovered high value. There are other points. Some environmental advocates see *ex situ* genetic preservation as undermining support for *in situ* conservation of nature in the wild. Many potential donors have guidelines that don't include support for this topic, and public agencies are constrained by their authorizing laws. Furthermore, current widespread fiscal and monetary fragilities are a drag on investment in new public enterprises.

The raw challenge posed by funding constraints and priorities is compounded for DNA Net Earth by the differing objectives of those who would be its primary advocates and participants. National sovereignty is a particularly significant complication, driven by politicized concerns over ownership of living resources. Various developing nations have restricted or threatened to restrict scientific collection of wild species, expressing concern that collectors from developed nations will commercialize products and not share revenues. Compartmented leadership and funding for collections is another issue. Food and agricultural interests are not well connected to those who associate their work with biodiversity. That loose connection is exhibited in government agencies,

private philanthropy, and academic institutions. In the United States, for example, the Agriculture Department is principally overseen by the Senate and House Agriculture Committees, whereas the U.S. Fish and Wildlife Service is overseen by the Natural Resources Committee in the House and the Environment and Public Works Committee in the Senate. In the United Nations system, the specialized agency addressing agriculture is the FAO, whereas biodiversity is addressed under the Convention on Biologicial Diversity and a host of other nature and wildlife oriented conventions, several with their own secretariats.

Another challenge is the tradition of curatorial independence in the natural history institutions where collections of most species are currently held and curated, generally in the form of remains kept dried at room temperature or stored in alcohol or formalin. The challenge is not a lack of support in principle. Preservation is a cause to which staffs of museums, zoos and botanical gardens naturally respond. The challenge, acute at museums, is that curators—who are research scientists—do not generally want to spend much time or commit resources on anything other than what they study. This inclination is reinforced by the omnipresent struggle for funding. Doing something different and without connection to ongoing research projects might be unappealing, but doing that without additional funding is anathema. In consequence, museum collections usually reflect interests and careers of curators rather than shared program or policy goals. On top of this, some curators are reticent to share anything—materials or information—even if the process is simple and at little or no cost. Some are likely motivated in this behavior because of concerns that others will benefit from their work in competing publications or will use materials or information to second guess their studies.

None of these challenges is insurmountable. Some returns on investment are immediate. Each species entered into storage immediately becomes a resource for research and applications and can have its DNA "barcoded" on accession for rapid identification, such invasive insect species or confiscated ivory. Furthermore, national and international agencies have long engaged in big projects without immediate returns, including manned space flight, colliders for high-energy particle physics and military defense. A shift in perception would be needed for DNA Net Earth to be seen in this league, but that may be feasible and the expense would be very small by comparison. It may in fact be possible to move DNA Net Earth forward with relatively modest new funding for the steps recommended: a website with key relevant information that is managed on-site by contributing facilities; new incentives and mandates for contributing information and conducting targeted research; and more public engagement.

Ignoring *ex situ* preservation as species go extinct in the wild is a weak premise for environmental advocacy. We can work to protect wild populations *and* to preserve DNA. National sovereignty issues can be addressed though agreements on legal ownership and profit-sharing by providing support for domestic storage, and by increasing trust through partnerships and mutual understanding. The institutional divide between agriculture and biodiversity is one that time and effort should shrink, and the rugged independence of museum curators is already diminishing because of the need to demonstrate social relevance to funders.

Many science agencies, organizations and publishers have already weighed in to promote data sharing (and prevent data hoarding). Some require sharing as a condition of support, including the National Institutes of Health (NIH), National Science Foundation (NSF) and Gordon and Betty Moore Foundation in the United States; and

the National Environmental Research Council and Wellcome Trust in the United Kingdom. Various journals, including Nature and Nucleic Acid Research, require authors to share data. Sharing specimens is just an incremental step beyond. One particularly intriguing model is "GenBank." GenBank is a NIH program that maintains an online database of publicly available DNA sequences by species. GenBank has become widely used by grantees and authors as a way to meet data sharing requirements and to get DNA sequence data. GenBank held about 200,000 DNA sequence records as of April 2011, and the database is growing rapidly. DNA Net Earth could connect to publishers and funders in a similar way for contribution of specimens and could link information on it to the GenBank database. All of the challenges above can be met, but progress requires that key questions be addressed and new steps taken.

Key Questions

What List Should be Used?

There is no authoritative list of the 1.9 million known species of organisms, despite the relatively manageable number and permanence of known species as contrasted with, for example, the 7 billion people now living on Earth. The lack of an all-species list is mostly a reflection of its independent underlying sources. Some contributing lists, such as the International Ornithologists' Union World Bird List,[13] are collaborative efforts by professional societies within taxonomic disciplines. Others, such as the World Spider Catalog by Norman I. Platnik of the American Museum of Natural History, are compiled by individual taxonomists who study particular groups. There is no all-species taxonomic authority.

Nevertheless, there are good efforts underway to compile all-species lists that derive from the underlying lists. Two of the best are the Integrated Taxonomic Inventory System (ITIS) managed by the U.S. Geological Survey and "Species 2000" managed at the University of Reading in the United Kingdom. Species 2000 has partnered with ITIS in developing a list named the "Catalogue of Life,"[14] which currently includes more than 1.3 million unique species names. The Catalogue of Life is the backbone list of names for the "Encyclopedia of Life" project, whose goal is to prepare a webpage of information for each known species, and it is probably the best current species reference list for DNA Net Earth. GenBank also provides a useful template. It approaches names flexibly, allowing those submitting sequences to assign a name that is referenced to multiple "out-link" taxonomic sources, which include the Catalogue of Life and other sources.

The bad news is that most species have not yet been described and that some will undoubtedly become extinct before their existence is recorded. The good news for implementing DNA Net Earth is that new species are being described at a manageable rate—thousands, not hundreds of thousands per year—and through formal, peer reviewed publications. The process should speed up with the advance of web-based publication, but it will still involve peer review, publishers and, usually, some kind of government or other funding support. One mechanism for preserving material from newly described species is for specimen contributions to be required by funders and by those who publish naming articles, as has been done successfully with sequence information provided to GenBank's DNA database. [15]

What Should be Kept?

An initial question is whether any biological material should be preserved, or whether the DNA of specimens should be initially sequenced and only the data stored. It is unproven, however, whether raw DNA can be used to produce an eukaryotic organism, or how much it would cost (estimates predict in the millions). Also, DNA is not the only information in organisms and cells, which have epigenetic factors and countless chemicals and structures whose link to DNA is equivocal or difficult to discern. In addition, although the cost of DNA sequencing has dropped dramatically, the current cost of sequencing an individual organism's genome is still much higher than freezing it, and frozen DNA can be sequenced in the future, almost certainly at a lower cost. Sequencing DNA is an important objective, but not as an alternative to its preservation.

Live Organisms and Frozen Viable Seeds and Cells

Living organisms are the gold standard for DNA Net Earth. Most botanic gardens, zoos and aquariums are attuned to the value of species diversity in holdings of living organisms. Mostly these institutions are limited in what they can hold by space and money. Live animals, especially, are expensive to keep.

Frozen viable seeds and cells of plants and animals are the silver standard for DNA Net Earth and provide the greatest opportunity for progress. Seeds are often abundant, typically compact, comparatively easy to freeze and store, and self-packaged for germination. Plant tissue cuttings for many species can be used fresh or after being frozen to propagate new whole plants. Viable animal sperm and eggs can also be kept frozen and combined later to produce fertilized eggs. The San Diego Frozen Zoo® has shown that viable animal cell cultures can be maintained frozen in scale. The zoo currently keeps 20,000 cultures covering 1,000 species and it estimates that a cell culture costs between $200 and $300 to establish and about $1 annually to maintain, well under the costs of live animal maintenance. Cloning animals from such viable cells, however, is a far less developed and practiced technology than for plants, although with promising beginnings.[16]

Cloning by nuclear insertion or induction of differentiated cells has to date been difficult and limited, and no one can predict with certainty how the technology will develop in the future. That said, if the future is measured by a century and more, it seems likely that there will be more widely available and less expensive technologies.

Additionally, frozen viable collections should, whenever possible, represent multiple individuals of a species. In essence, they are frozen populations just as living populations exist in the wild, and captive and cultivated populations live in zoos and botanical gardens. On average, the Frozen Zoo® currently maintains 20 samples for every species that it holds. This is important because frozen populations—like the living—provide genetic diversity for adaptive traits, and larger populations pose less risk that deleterious genes will be expressed in offspring. Small population size and inbreeding is a bane for endangered species, which zoos and frozen cell cultures can counter by augmenting genetic diversity.

Live organisms and frozen viable seeds and cells should be treated as preferred but nonexclusive items in a portfolio of preservation options, and the relatively costly maintenance of live animals and viable animal cell cultures should be focused on priorities such as endangered species conservation and representation of higher taxonomic groups such as families or genera. Nevertheless, collection and frozen storage of viable animal cells

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for any species not adequately represented should be considered in all-species "grassroots" initiatives, and a current lack of funds or technology for establishing cultures does not mean viable cells should not be preserved for potential culturing in the future. In the case of small animals, such as insects, this can be whole animals in a vial.

Dead Cells with Sequenceable DNA

The value of viable cell cultures notwithstanding, many frozen and room-temperature collections include specimens of non-viable (read "dead") cells. The Smithsonian Institution's new cryogenic biorepository is primarily expanding its limited current collection with frozen tissue kept by its curators and collections managers in their own labs. Some of these tissues will have viable cells but most will not. The Smithsonian's goal to freeze 50 percent of genera by 2018 is for dead cells, with any live cells included as a bonus. The American Museum of Natural History in New York is accessioning both dead and viable cells into its frozen animal tissue collection. Most other facilities storing frozen animal tissue take the same approach. The San Diego Frozen Zoo® is one of very few maintaining viable animal cell cultures for biodiversity conservation.

Freezing dead cells is not a bad idea. It is more convenient and cheaper for collectors in remote places to kill animals like fish, frogs or ants and bring specimens back at room temperature than it is to freeze them on the spot and keep them frozen all the way home. Also, collection is often done without preservation of viable cells in mind or by local collectors without reagents or equipment for freezing. The DNA of dead cells may remain largely intact depending on time and conditions before freezing. Indeed, under favorable ambient conditions, the cells of a just departed animal may remain alive for days or even a few weeks. Freezing dead whole organisms or cells with still intact DNA is the bronze standard for preservation, not the best but still a winner. With time, money and new sequencing technology that can evaluate partially degraded DNA, it has even been possible to reconstruct the genomes of extinct species from animals found in circumstances favorable to preservation where they died long ago. This has been done for the mammoth and Neanderthal. If the DNA of these can be sequenced, so might the DNA from at least some species in the vast dried and otherwise preserved collections of natural history museums, side-stepping the need for recollection of fresh specimens insofar as DNA sequence information is the objective. Furthermore, cloning is only one objective. Indeed, it is unlikely that more than a small fraction of known species will be cloned in the future even though the *potential* for cloning any species as needs are identified is a central purpose of DNA Net Earth. Once DNA is sequenced its genes can be identified and studied and the products of gene expression examined for values. One gene may turn out to be just what a

related endangered species needs to survive or research may determine that another gene upon insertion will alleviate a disease in man.

However, time and money cut both ways in deciding whether to preserve viable or dead animal cells. Culturing and cloning from viable cells is now straight-forward for some species and the cost is measured in thousands of dollars, rather than the millions along with technical uncertainties and time that would be required to clone a species such as the Asian gaur using DNA from dead tissue. Cloning from dead cells would require sequencing their DNA, synthesizing matching DNA and then situating the new DNA in a nucleus within an egg or other undifferentiated cell in such a way that cellular mechanisms for gene expression work, and the cell divides and develops. This has been done for the bacterium *Mycoplasma mycoides,*[17] but it lacks a nucleus and has a tiny genome. Furthermore, an individual of each sex would be required for "natural" reproduction to follow in species that have sex, and inbreeding in the progeny of such a pair would challenge population fitness and growth. Genetic engineering, breeding strategy and supportive, compensating environmental regimes might fix adaptive frailties, but would be difficult.[18]

Extracted Sequenceable DNA

DNA can be extracted from cells, stored, and replicated without diminishing the source. The extracted DNA can be frozen, and efforts are being made to establish conditions for room temperature storage that provide long-term stability that matches freezing, with hoped for lower cost and ease of handling. Yet, DNA is not the only information about a species in the whole organism, its tissues, or its cells, albeit the most important. DNA Net Earth should encompass a full portfolio of preservation choices including living organisms, frozen viable seeds and cells, dead cells with sequenceable DNA, and extracted DNA.

How Should Specimens Be Kept?

Zoos, aquariums and botanical gardens have mountains of experience and information on keeping live organisms, backed by reams of policies for animals and active discussions on unmet needs. ISIS, for example, maintains a Zoological Information Management System, which it describes as "a real-time web-based institutional and pooled animal and veterinary knowledgebase."[19] One can stipulate that *ex situ* maintenance of living plants and animals is well studied and effectively addressed.

New frozen collections are central to development of DNA Net Earth and their management varies. In the case of liquid nitrogen storage, the samples are typically kept at a temperature of -160 °C or cooler, in vials that are placed

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in racks that hold them in vapor above the liquid or in the liquid directly. The temperature of storage in electrical freezers varies. Some animal tissues are kept at -80 °C (e.g. Academy of Natural Sciences and FisherBioscience collections). Plant seed banks generally set temperature at -18 °C or lower (e.g. Svaldbard Global Seed Vault), which the FAO and other plant genetic resource authorities have endorsed for long-term seed conservation and which can be achieved by conventional freezers. Managers of liquid nitrogen facilities make a good case that their facilities are colder, not subject to loss of power and less expensive than electrical freezers. There are also opportunities for innovation with liquid nitrogen, for example in development of solar-powered, self-contained, low-maintenance liquid nitrogen generators that can be installed in remote locations. However electrical freezers are widely used and many will not be replaced with liquid nitrogen anytime soon. As noted above, ambient temperature storage of extracted DNA is being investigated actively and may prove to be an important complement to frozen storage.

Despite great effort and investment, most collections have not lasted when time is measured in millennia or even centuries. The ancient library at Alexandria is just one in a series of losses through the likes of accidental fires, floods, storms, pests, theft, civil disorder, war and budget deficits. Yet DNA Net Earth should be designed to last for millennia. To that end, it should be "distributed," with collections maintained at several locations. It should aim to locate at least one collection facility in each sovereign nation to address patrimonial concerns and to facilitate local participation in collecting, research, conservation and education. It should also aim for specimens of each species to be kept in at least one location at a facility overseen by an international regime with committed administrative and financial support.

How Should Specimens be Exchanged, Used, and Owned?

The prime imperative of DNA Net Earth is long-term preservation, but consistent with that purpose it is critical that specimens be available for exchange and use in research and applications. Living plants, viable seeds and tissues are exchanged by botanical gardens as a matter of course for the benefit of preservation and research, and with no depleting effects. Zoos are advanced in exchanging gametes and live animals for breeding, which augments and improves captive populations overall. More generally, exchange and use should be strongly encouraged when specimens are not exhausted or collections can be replenished readily by reproduction or additional collecting.

Depleting uses for noteworthy research and applications should also be facilitated so long as adequate material is kept for preservation. Viable cells cultures can replenish themselves for some generations or, in some cases, indefinitely,[20] so some cells can be taken without diminishing collection size. The San Diego Frozen Zoo® uses its collection for research advancing conservation of endangered species. Consider the use of *Thermus aquaticus*, a thermophillic chemosynthetic bacterium discovered in a hot pool in Yellowstone National Park. A culture collection was established for it at the ATCC. Research on the species isolated an enzyme, *Taq polymerase*, which retains its catalytic functions at high temperatures, and the enzyme became the foundation element of the polymerase chain reaction ("PCR") process now widely used to replicate DNA.

Dead cells, however, cannot replicate and can present collection managers with tough decisions. A memorable illustration concerns a collection of hair at the Academy of Natural Sciences in Philadelphia that include samples

from the first 12 U.S. presidents, some taken in life and some later on. To no surprise, requests have been made for samples to sequence. However there are complications. The hair samples do not include follicles—they were sniped not yanked—and strands without follicles contain little DNA, most of it from mitochondria. Sequencing the mitochondrial DNA of a president's hair could still be interesting for students of presidential history, but it would only examine the president's maternal ancestry and not to the president's offspring, because sperm do not contain mitochondria, which are passed from generation to generation only by women through their eggs. Another issue is its age because the DNA degrades over time. The academy has kept hold of its hairs so far, but as sequencing technology improves and less material is required, the academy might just give up a hair or two for the right proposal. In fact, there is some nuclear DNA in mammalian hair, and it might just be possible to confirm whom the presidents fathered after all. This same issue, not usually involving presidents, comes up all the time at museums with old collections. Often there is enough material to give a strand or slice without regrets, but not always.

Whether or not specimens are live or dead, ownership matters in considering their use. The specimens kept in a facility may be owned by the facility itself, by donors or by a third party. Collection facilities are obligated to honor the conditions they accept on accession, but sometimes they do not. Donors retaining ownership or requiring conditions should help to meet storage and administrative costs, but not all of them do. Ownership is particularly sensitive at the international level because of developing nation concerns that native specimens will be taken into laboratories of developed nations, analyzed and commercially exploited. In consequence, some developing nations have on occasion prohibited specimen-collection except for domestic analysis and storage. The CBD parties adopted a protocol in 2010 designed to defuse the issue by requiring prior informed consent from countries of origin for access to and benefit from collection of native species.[21] This Nagoya Protocol may help, but it is not yet effective[22] and the combined debating points of sovereignty, heritage, patrimony and colonialism require case-specific, politically attuned, pragmatic attention. Concerns that developed nations will commercialize species without remuneration can be addressed by conditions in collection or export permits that provide for revenue sharing with countries of origin. Collection or export permits may also require samples of collected specimens to be deposited in domestic facilities. Certain facilities participating in DNA Net Earth might incorporate representatives of concerned developing nations into their governance structures. No less important than legal instruments, long-term working partnerships are needed to build trust.

How Should Specimens Be Collected and Transported?

Collection must be coordinated with recipient facilities and be consistent with the preservation option planned. Zoos, aquariums and botanic gardens generally closely oversee the collection and transport of living whole organisms, and seeds typically remain viable at least long enough to reach freezers alive. However, animals collected for viable cell cultures usually must be brought back alive or killed and frozen in the field, whole or in pieces, and transported home in insulated containers.

Expanding public engagement could significantly advance collection of specimens for frozen collections, especially for seeds, and for the insects and other invertebrate animals that constitute most known species. Another

plus for these species is that collection may not require permits and unseasoned collectors are less likely to deplete populations, get seriously bitten, or be zoonotically inflected. Facility-specific guidance will be required for permit, collection and transport needs, and mechanisms will be needed for identification and other quality control. However, public engagement in collection can offer multiple benefits by engaging volunteer or low-cost labor and advancing public appreciation and support for the initiative. A public engagement initiative would seek to tap the same interests that lead individuals and organizations to keep track of species in geographic areas (e.g. backyards, parks, refuges, private land trusts, counties, states and nations). It could be pursued through "citizen science" projects involving students and adult volunteers as are currently offered on other topics (e.g. bird and frog counts) by various nonprofit organizations, government agencies, museums, science centers and schools. DNA Net Earth also could work to help local collectors in developing countries secure development assistance funding or funding built into research grants.

The principal challenge to public engagement is identification and other quality control—the concern that specimens will be unwanted by storage facilities, misidentified or mishandled on their way to storage. Museums often decline to accession "walk-in" specimens because they are not priority species for curation effort or use of limited storage space. However, this challenge can be managed if storage facilities and the local nonprofits, museums and agencies involved plan ahead and work with collectors. All could be informed by a DNA Net Earth website. There are many options. Teams of volunteer collectors could be supervised by individuals with training and collection permits as needed. Storage facilities or museums could sell collection kits, including what is needed to freeze specimens. The specifics of citizen collection will vary with place, species collected, storage facilities and other factors, but it should be possible for DNA Net Earth to help develop tools for the work that can be tailored to these situations. Besides providing free labor, citizen science could break down or at least walk around the barriers that currently limit many collections to species involved in selected research and could engage public agencies and secure public funding.

Three Steps Forward

One: Establish, Develop and Maintain a DNA Net Earth Website

An upfront, fundamental need for launching DNA Net Earth is development and maintenance of a website that connects diverse relevant institutions and programs, and provides information and communication vehicles facilitating progress with minimal top-down decision-making. The site should be as self-executing as possible to promote early contributions by collectors and facilities, and to continue growth in species coverage. *In particular, information on facility holdings, modes of storage and other facility information should be posted directly on the website by facilities after registration with the website and without prior review by website managers.*

The website design should draw from the expertise of enterprises engaged in distributed, web-based management of geo-referenced information, such as the Wikipedia Foundation, Google and Esri, and should optimize integration with related-websites, including the DNA Bank Initiative, GenBank, the Catalogue of Life, Encyclopedia of Life and Wikipedia. To accomplish this breadth, the website team will need leadership that is fluent in

An upfront, fundamental need for launching DNA Net Earth is development and maintenance of a website that connects diverse relevant institutions and programs, and provides information and communication vehicles facilitating progress with minimal top-down decision-making.

the work of relevant institutions and programs, but also brings transcending perspectives and information skills, such as expertise in Wiki information systems and public engagement.

The website team could be selected through an evaluative process responding to a request for proposals. The RPF could call for a website design that provides access to information by species or registered facilities, with ways to combine information in useful categories such as countries or higher taxonomic groups. One task specified in the RFP should be to describe how the website can be maintained, marketed for use to facilities and others, and managed and housed long term.

The website should include the following at a minimum:

- A "backbone" reference list of described species. The Catalogue of Life list is the apparent best choice. It can be used now, but needs to be completed and kept up to date as new species are described.
- Species and estimated numbers of samples or specimens at facilities, sortable by the mode they are kept, including:
    i. living plants
    ii. living animals
    iii. viable frozen seeds
    iv. viable frozen cells (other than seeds)
    v. frozen dead cells with sequenceable DNA
    vi. extracted preserved DNA and mode of preservation
    vii. any DNA sequence records, which could be provided by links to GenBank.
    viii. ambient-temperature dead cells whose DNA may or may not be sequenceable (this should be provided as an option for facilities and could be pointed toward priorities such as type specimens or endangered or extinct species).
- Whether additional specimens will be accepted by facilities and requirements for submission of specimens including collection protocols, and points of contact for questions and coordination.

- Additional information that facilities may want to provide, such as information on research, applications, and costs.
- General guidance and useful information on collection and storage, in terms that are understandable to a range of individuals using the site.
- General information facilitating the use and exchange of stored materials consistent with long-term maintenance of adequate collections.
- Other useful information on research, applications, or other matters.

Two: Establish New Incentives and Mandates for Contributing Specimens

In conjunction with website development, supporters of DNA Net Earth should invest in new incentives and mandates for contributing specimens, and for targeted research and applications. This could be done through support to nonprofit organizations (including research and educational institutions as well as policy groups), and could include:

- Collaborating with government agencies to develop policies requiring specimen contributions, such as conditions attached to collection or export permits or requirements for research funding. Those involved in this should also work to improve policies for use, exchange, and ownership.
- Collaborating with publishers of taxonomic research in order to require specimen contributions to facilities before articles naming new species are accepted for publication. Grants for this work could, in principle, be made directly to the publishers as well as to those seeking to influence them.
- Direct funding of institutions engaged in collection and in keeping specimens, including work to enhance collection and specimen management in countries where such activity is underrepresented.
- Targeted funding of relevant research and applications such as field collecting techniques for frozen storage, stability of preservation at different temperatures and conditions, cloning, and applications for societal priorities such as food security, health, energy, waste management, and biodiversity conservation.

Three: Advance Public Engagement

Support could be provided to advance "citizen science" and other public engagement in collecting specimens for facilities, including mechanisms to assure accuracy in identification and in quality control for transport. This should address funding needs of both the collectors and those experts who are needed to identify specimens, to assist with collection methods, and to manage specimens (e.g. natural history museums). Many curators and collection managers already do public service of this kind to some degree, but new incentives for their engagement are key to addressing concerns over identification, quality control, and permits. If that is provided, public participation could ramp up quickly with support to groups already engaged in "citizen science," such as the National Wildlife Federation and Audubon Society in the United States, which have international collaborators.

The great natural history museums of the world all hold remnants of species in storage cabinets that are not yet described and are extinct. Wild nature holds many more of such unknowns. DNA is the information that fundamentally makes them what they are and is of greatest value to man. We shouldn't let it slip away.

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Endnotes

1. The literature on climate adaptation is large and growing. Recent Brookings Institution reports address, for example, the Green Climate Fund, the imperative of adaptation, and "green growth" generally, searchable at www.brookings.edu.

2. Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B (2011). How Many Species Are There on Earth and in the Ocean? PLoS Biol 9(8).

3. Hawksworth DL (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycological Research: Vol. 95, Issue 6, pages 641-655.

4. Schloss PD and Handelsman J(2004). Microbiol Mol Biol Rev. 68(4): 686–691.

5. Statistics are from the IUCN Red List of Threatened Species (2010) unless otherwise noted. Bacteria are from J.P. Euzéby at http://www.bacterio.cict.fr/number.html. Fungi are from David L. Hawksworth. J. Mycological Research (2001). New species are described continually, so these numbers are approximations and overly precise.

6. Protists are microbes that have true nuclei ("eukaryotes") unlike bacteria ("prokaryotes") but lack the specialized tissues found in plants, animals, and fungi. Most protists are unicellular (the protozoans) but some are not. The amoeba and paramecium are protists. Protist taxonomy is a unsettled.

7. Published by the United Nations Environment Programme (UNEP).

8. The CBD parties, for example, have identified "climate change and biodiversity" as the "new great threat to biodiversity" (www.cbd.int/climate/), with concerns ranging from alpine plants with no where to go for cooler temperatures to marine corals whose exoskeletons may not form in a more acid sea.

9. Recognizing that some previously described species will elude re-collection.

10. http://www2.isis.org/AboutISIS/Pages/About-ISIS.aspx.

11. Room temperature storage is being studied for dead cells and extracted DNA, and would significantly reduce cost if long-term DNA stability is provided.

12. See http://www.atcc.org/About/WhoWeAre/tabid/139/Default.aspx for this information on the ATCC. The FisherBioservices information is a personal communication from Dr. Kathleen Groover.

13. The World Bird List website cites an "old Chinese proverb" as saying "Wisdom begins with putting the right name on a thing."

14. http://www.catalogueoflife.org/

15. Obviously fossil species lacking tissue would be an exception.

16. One approach is to remove the nucleus from a viable cell and insert it into an egg of the same or a closely related species whose own nucleus has been removed. Under the right conditions, which vary with species and can be difficult to establish, the egg will divide and develop into a clone of the individual whose cell nucleus was inserted. This is how Dolly the sheep and various other animals have been cloned. It has also been shown possible to clone an animal by prompting division of viable "totipotent" (or "fully pluripotent") stems cells separated from early stage embryos or, as reported for mice, artificially induced (see MJ Hazen and others published online May 20 2010 Science 2 July 2010: Vol. 329 no. 5987 pp. 52-56 DOI: 10.1126/science.1190719).

17. Published Online May 20 2010 Science 2 July 2010: Vol. 329 no. 5987 pp. 52-56 DOI: 10.1126/science.1190719

18. Not to mention, if an individual were a Neanderthal, should the child be sent to school or to a zoo?

19. http://www2.isis.org/products/Pages/default.aspx

20. And extracted DNA can be replicated without limitation.

21. The Nagoya Protocol, adopted at the 10th Conference of the Parties to the CBD in Nagoya, Japan, in October 2010.

22. As of 6 December 2012, the Nagoya Protocol had been ratified by India, Jordan, Mexico, Rwanda, and Seychelles, accessioned by Ethiopia, Fiji, and Laos, and accepted by Gabon. The protocol will not enter into force until 90 days after the 50th instrument of ratification, acceptance, approval, or accession is deposited (Article 33).