Genomics and proteomics in bioarchaeology - Review

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Abstract

The recent technological developments have allowed to use molecular-biology tools for archaeological studies. This way, some ancient nucleic-acid and peptide remains can be analyzed with an unprecedented resolution power. Thus, the second-generation DNA sequencing technologies have allowed to sequence ancient genomes for the first time, which has revealed interesting facts about the evolution of different species. This way, it has been found that our ancestors inbred with Neandertals and Denisovans, since some current human populations carry part of their genomes. Additionally, the third-generation sequencing of nucleic-acids holds the promise of direct ancient-RNA sequencing, without a previous cDNA synthesis, which would open the door to transcriptomics of ancient RNA. The nucleic-acid sequencing is faster and cheaper than the peptide sequencing, generating longer contigs after the assembly of reads. Yet, the former molecules degrade much faster than the latter, and therefore the peptide sequencing has become a powerful tool in bioarchaeology. This way, it has been demonstrated that the birds are indeed feathered dinosaurs. Finally, the prospect of bringing "back to life" some extinct species by means of synthetic genomics, reverse-engineering current genomes and cloning ancient species is certainly exciting and challenging.

Key words: paleogenomics, paleotranscriptomics, paleoproteomics, paleontology, paleobiology, paleomicrobiology, paleobotany, paleozoology, zooarchaeology, paleoecology, paleogenetics.

Resumen

Los recientes desarrollos tecnológicos han permitido aplicar herramientas de biología molecular a estudios arqueológicos. De este modo, algunos restos de ácidos nucleicos y péptidos antiguos pueden ser analizados con un poder resolutivo sin precedentes. Así, las tecnologías de secuenciación de ADN de segunda generación han permitido secuenciar genomas antiguos por primera vez, revelando hechos evolutivos interesantes sobre distintas especies. De este modo, se ha encontrado que nuestros ancestros se cruzaron con los neandertales y denisovanos, ya que algunas poblaciones humanas portan parte de sus genomas. Además, la secuenciación de ácidos nucleicos de tercera generación podría permitir secuenciar directamente ARN antiguo, sin síntesis previa a ADNc, abriendo las puertas a la transcriptómica de ARN antiguo. La secuenciación de ácidos nucleicos es más rápida y barata que la de péptidos, generando "contigs" más largos tras el ensamblaje de las lecturas. Sin embargo, las primeras moléculas s degradan mucho más rápidamente que las segundas, y por tanto la secuenciación de péptidos representa una poderosa herramienta en bioarqueología. De este modo, se ha demostrado que las aves son dinosaurios con plumas. Finalmente, la posibilidad de "retornar a la vida" a algunas especies extintas mediante genómica sintética, ingeniería reversa de genomas actuales y clonación de especies antiguas es ciertamente un excitante reto.

Palabras clave: paleogenómica, paleotranscriptómica, paleoproteómica, paleontología, paleobiología, paleomicrobiología, paleobotánica, paleozoología, zooarqueología, paleoecología, paleogenética.

Introduction

The archaeology studies the past through its remains. On the other hand, the molecular biology studies the biological entities, including acellular ones (virusoids, viroids and viruses) and organisms based on living cells (prokaryotes and eukaryotes), as well as their remains. A few years ago, the archaeology and the molecular biology could not interact, simply because appropriate technology was not available for the latter to study the sources provided by the former.

Such scenario changed with the development of new molecular-biology methodologies, allowing to study the nucleic acids and peptides (like proteins) of archaeological remains at the molecular level. This new multidisciplinary research area corresponds to ancient genomics, ancient proteomics, bioarchaeology, biomolecular archaeology, molecular paleontology, molecular paleobiology, molecular paleomicrobiology, molecular paleobotany, molecular paleozoology, molecular paleoecology, paleogenetics, paleopopulation genetics, paleogenomics and paleoproteomics, among other terms (Becker, 1999; Lambert et al, 2002; Larsen, 2002; Paabo et al, 2004; Gugerli et al, 2005; Dorado et al, 2007, 2009; Peterson et al, 2007; Hofreiter, 2008; Knudson and Stojanowski, 2008; Millar et al, 2008; Schlumbaum et al, 2008; Vigne and Darlu, 2008; Brown and Brown, 2011; DeBruyn et al, 2011; Jones, 2011; Lalueza-Fox and Gilbert, 2011; Stoneking and Krause, 2011; Disotell, 2012; Eriksson and Manica, 2012; Hofreiter et al, 2012; Huynen et al, 2012; Kirsanow and Burger, 2012; Matisoo-Smith and Horsburgh, 2012; Rizzi et al, 2012; Smejkal et al, 2011; Shapiro and Hofreiter, 2012; Wales et al, 2012; Wall and Slatkin, 2012; Campana et al, 2013; Dabney et al, 2013; Green and Shapiro, 2013; Kruger, 2013; Molak et al, 2013; VanArsdale, 2013).

This new scenario has made possible that some scientific publications that had been traditionally indexed in the Social Sciences Edition of the Journal Citation Reports (JCR) of the Web of Science (WoS) - Web of Knowledge (WoK) of Thomson Reuters, are now also indexed in the Science Edition <http://apps.webofknowledge.com>, as is the case of the *Journal of Archaeological Science* <http://www.journals.elsevier.com/journal-of-archaeological-science>.

The possibilities of these scientific interactions are fascinating. They have the potential not only to study the living entities of the past, reading genetic information stored in their molecules, but also to clone them and even “bring them back to life” again in some instances. These proposals can be considered as a scientific challenge and goal per se (for the advancement of science and human knowledge), but at the same time they may represent valuable tools to reverse extinction events in some cases. This review highlights such ideas in the context of the evolution and mass extinctions, taking into account the nucleic acid and peptide sequencing and the potential of cloning extinct species.

Evolution and mass-extinctions

The Earth is a fertile planet filled with biological entities. This has been possible due to several remarkable factors. Thus, the physical and astronomical peculiarities of the Earth and the Solar System allowed the spontaneous generation of life about four milliard years (Mdy) ago. Subsequently, the life forms proliferated, diversified and colonized virtually all the available environments, creating different ecosystems. Neither

the Earth nor its biological entities are static; on the contrary, they are dynamic and thus continuously changing. The ecosystem environments change (eg., climate, geographical position, etc) and the biological-entity genomes mutate both spontaneously (eg., DNA polymerase errors), as well as due to DNA damage and wrong repair. The development of sex in the eukaryotes about one Mdy ago, and particularly the homologous-chromosome crossing-over during meiosis, was also a key strategy to generate biodiversity without the deleterious risks of the mutations.

This has two consequences. On one side, the natural populations of biological entities are not usually monomorphic (genetically identical clones), but polymorphic (biodiverse). Obvious exceptions are the eukaryotic populations that only reproduce vegetatively, without sexual events, but even then, some mutations and variability may also arise. The other biological entities (virusoids, viroids, viruses and prokaryotes) are usually quite polymorphic, due to DNA-polymerase errors which are later on wrongly repaired, and thus fixed as mutations. On the other side, these biological changes at the genomic level allow the generation of varieties of the same species, then subspecies and eventually different species, given the other speciation requirements, like the spatial separation. Therefore, all living entities have a common ancestor, as shown by the dendrograms or phylogenetic trees that graphically depict the biological evolution (Fig. 1).

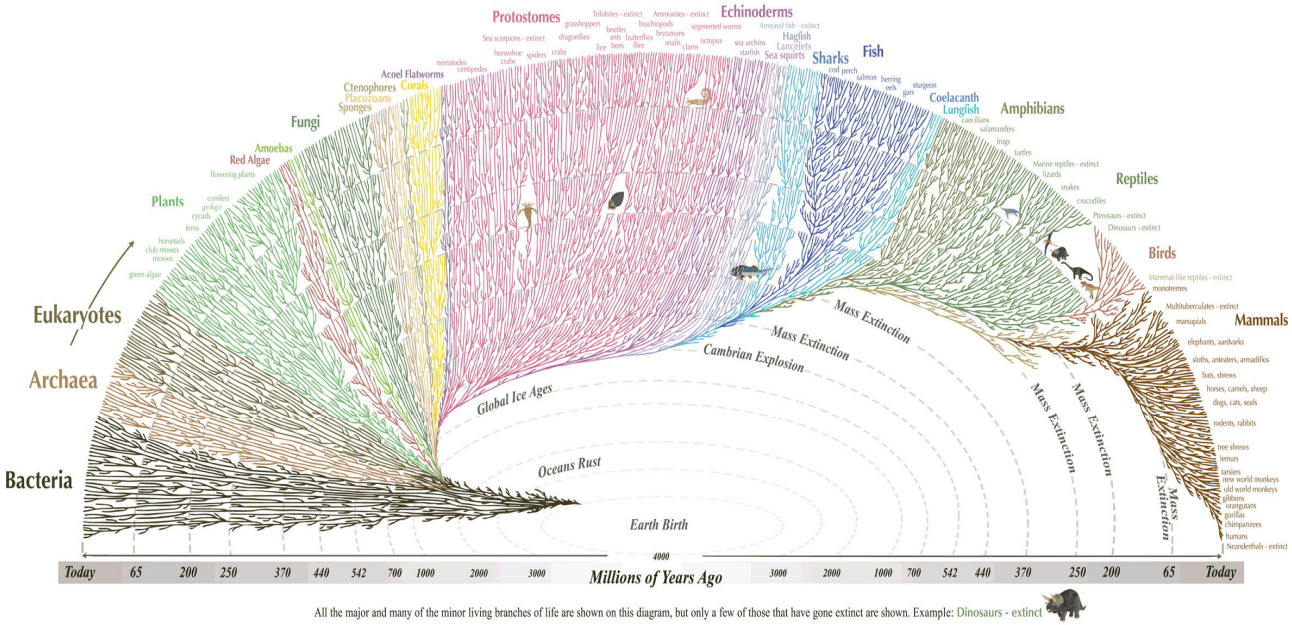

Figure 1. The tree of life. Phylogenetic tree of the living species, showing also a few extinct ones as examples. Figure credit: The Great Tree of Life. © 2008 Leonard Eisenberg. Evogeneao <http://www.evogeneao.com>.

In turn, such biodiversity may play a crucial role as a strategic survival insurance for the species, albeit not being 100% efficient. Indeed, as the Thomas Robert Malthus showed, the food resources increase in an arithmetic (linear) fashion, whereas the populations grow in a geometrical (exponential) way. As the naturalist Charles Robert Darwin exposed, this generates a significant struggle of the living species, with the survival of the fittest (term coined by the researcher Herbert Spencer), and therefore a natural selection that drives the evolution of the species. This is possible due to the genome biodiversity of the populations forming such species. In other words, only the

individuals that can adapt to the changing world will survive and have offsprings or descendants, which will inhering their progenitor's genomes, adding to them more biodiversity in each generation.

Yet, even though the different species try to adapt to the changing environments, the environmental variations may be so drastic and quick over the course of the geological ages, that some of the living entities may not be capable of adapting to such changes or may not move to other more favorable areas, and therefore they may suffer extinction. Indeed, it is considered that a mind-blowing 99% of all the species that lived on the planet Earth, at any time, are now extinct, which is both shocking and revealing (Dorado et al, 2010).

Nucleic-acid sequencing

The main problem of the current nucleic-acid-sequencing technology is that it is not possible to read long molecules in a continuous way. Instead, small reads are produced, which requires special bioinformatics tools to assemble the resulting conundrum into contigs, chromosomes and genomes. The near-future sequencing platforms promise much longer reads, albeit still being far off the desired large-full-genome lengths.

The nucleic acids can be read using the first-, second- and third-generation sequencing platforms (Dorado et al, 2008). The first-generation sequencing has a low yield and is the most expensive per sequenced base, albeit generating longer reads (~500 to ~1,000 bases), as we have optimized (Lario et al, 1997).

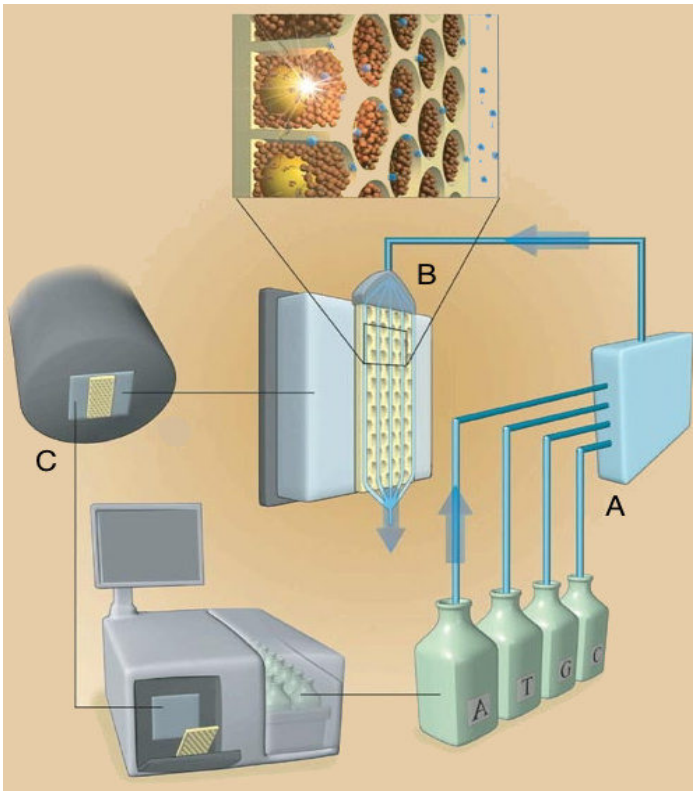

Figure 2. DNA sequencer. The sequencing-reaction chemicals are delivered (A) to a microfluidics chamber (B) and the light-signals generated are captured by a Charged-Coupled Device (CCD) camera and analyzed by a computer (C). Figure credit: GS FLX+ System, 454 Sequencing, 454 Life Sciences. © 2005 Roche <http://www.rocheinstitute.com>.

The second-generation sequencing (Fig. 2) increases the throughput and lowers the pricing per base several orders of magnitude, albeit generating shorter reads (~75 to ~800 bases). Finally, the third-generation sequencing allows to read single nucleic-acid molecules, without previous amplifications (Ginolhac et al, 2012). This latter approach can increase even further the throughput, reducing the price per sequenced base, and –most importantly– the length of the reads, promising even Megabases (Mb) of continuous reads in the near future. The second- and the third-generation sequencing of nucleic acids are sometimes called next-generation sequencing, yet such ambiguous naming should be avoided for obvious reasons (there will be always a next-something of anything, which basically means nothing!). Other advancements include the target-sequence capture and enrichment (Briggs et al, 2009), and the construction of single-stranded libraries (Meyer et al, 2012), among other developments. For more information, see the reviews of Rizzi et al (2012) and Orlando et al (2013).

On the other hand, the prospect of sequencing ancient DNA (aDNA) from fossilized material is exciting. Thus, such research were reported in the best scientific journals, including the results obtained from the Polymerase Chain Reaction (PCR) amplification and sequencing of DNA from magnolia leaves from the Miocene date 18 million years (My) ago, *Tyrannosaurus rex* bones from the Jurassic (80 My) and halite halobacteria from the Permian (250 My) and Silurian (425 My). The hematophage arthropods captured in amber are particularly exciting in this respect, since they may contain blood from their hosts, like the dinosaurs! Yet, unfortunately and shockingly, it was later on demonstrated that such amplifications were in fact due to modern-DNA contamination in most if not all the cases. The results are controversial for the bacterial aDNA amplification, with is still open to debate in the scientific community. In any case, the failure to amplify old nucleic acids can be explained by the following facts:

i) The absence of DNA repair after the death, degradation due to both endogenous and exogenous (microorganisms) nucleases, amine-group hydrolysis (depurination and strand breakage), cross-linking, chemical change of nitrogenous bases (tautomerization), etc.

ii) The DNA degradation increases with the molecule length and time, but mostly depends on the taphonomic and diagenetic history of the sample (mainly, the humidity and temperature, which favor the hydrolysis reactions).

iii) The RNA is never repaired and degrades much faster than the DNA, due to its higher chemical lability.

The degradation of aDNA has been recently studied by sequencing mitochondrial DNA (mtDNA) sequences of 158 moa bones from New Zealand, which were dated using the radiocarbon methodology (Dorado et al, 2012). This way, it was found that the mtDNA half-life was ~521 years, which means that aDNA can be amplified back to ~1’5 My. It should be also taken into consideration that the nuclear DNA (nuDNA) degrades at least twice faster than the mtDNA (Allentoft et al, 2012; Millar and Lambert, 2013), since the former is linear (instead of circular), and therefore it is susceptible to exonuclease degradation, being also less-protected (the nuclei envelopes are larger and more fragile than the ones of the smaller mitochondrial organelles).

Besides the controversial case of aDNA from bacteria (which could be preserved in the dry environment of halite minerals), some aDNA from other species has been

successfully sequenced, including mummified human remains of the Tutankhamun pharaoh of more than 3,000 years (y) ago, mammoths (20,000 y) (Poinar et al, 2006; Miller et al, 2008), Neandertals and Denisovans (40,000 y) (Krings et al 1997; Green et al, 2006, 2009, 2010; Noonan et al, 2006, 2010; Dodge, 2012; Meyer et al, 2012; Paixao-Cortes et al, 2012; Sankararaman et al, 2012; Lowery et al, 2013; Wall et al, 2013), bears (40,000 y) (Barnes et al, 2002; Noonan et al, 2005), rhinoceros (70.000 y), mastodon (50,000 to 130,000 y) (Rohland et al, 2010), cave bear (more than 300,000 y) (Dabney et al, 2013) and horses (560,000 to 780,000 y) (Orlando et al, 2011, 2013; Millar and Lambert, 2013), among others.

Interestingly, the second-generation sequencing technology is so powerful that it allowed to sequence for the first time aDNA genomes from hominids. Thus, the results obtained with the Neandertal and Denisovan genome sequencing have shown that we share genome sequences with them; there are Neandertal and Denisovan genes in our genome! (Sankararaman et al, 2012; Lowery et al, 2013; Wall et al, 2013). In other words, we have interbreed, generating fertile offsprings, and therefore all three are subspecies of the same *sapiens* species. The correct scientific name being, therefore, *Homo sapiens neanderthalensis*, *Homo sapiens denisova* and *Homo sapiens sapiens*.

The third-generation nucleic-acid sequencing also holds the promise of ancient RNA (aRNA) sequencing, since such technology does not require the previous nucleic-acid amplification. So far, the second-generation sequencing technologies have been used to sequence complementary DNA (cDNA) synthesized from aRNA isolated from corn (723 y) (Fordyce et al, 2013).

Peptide sequencing

In general, it is more difficult to analyze peptides (like proteins) than nucleic acids. This is aggravated by the fact that the peptides cannot be cloned and amplified as the nucleic acids (PCR, molecular cloning, etc). In general, the peptide sequencing is more expensive and time-consuming, generating shorter reads. Thus, the first-generation of peptide sequencing (Edman) allows to read about 30 amino acid residues. The second-generation of peptide sequencing (tandem mass-spectrometry) has a higher productivity, allowing to sequence any peptide when broken into pieces. Yet, the identification of the peptide reads may be arduous, requiring the use of previously-generated standard databases, which may significantly hinder such an approach (Fig. 3).

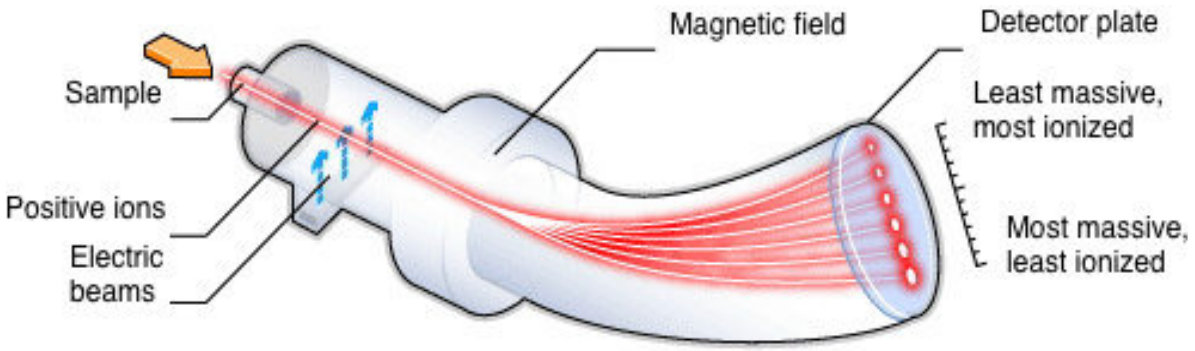

Figure 3. Peptide sequencing by tandem Mass-Spectrometry (MS/MS). The samples is injected, vaporized and ionized by electric beams, accelerated by a magnetic field, separated and detected. Figure credit: Mass Spectrometry. © 2006 Jiang Long, The Science Creative Quarterly <http://www.scq.ubc.ca/mass-spectrometry> and Creative Commons <http://creativecommons.org>.

Although the peptide sequencing is usually less convenient than the DNA sequencing, it has a critical advantage when applied to ancient remains: the peptides are usually much more stable than the nucleic acids, and therefore can be used when the latter fails. As an example, the NH_2-GPSGEOGTAGPOGTOGPQGLLGAOGFLGLOGSR-COOH peptide present in domestic sheep has been used as a molecular marker in zooarchaeological studies, allowing to properly identify previously and erroneously-classified goat bones by morphological methods (Buckley et al, 2010).

Additionally, although it is virtually impossible to sequence DNA older than one to two million years (with the possible exception of DNA preserved in salt, as previously discussed for bacteria), it is indeed possible to sequence peptides of such age and even much older. Indeed, the peptide sequences of different species, including dinosaurs, lizards, alligators, chickens, mammoths, etc have been obtained (Organ et al, 2008; Schweitzer et al, 2009; Smejkal et al, 2011; Cappellini et al, 2012) and the results have been surprising: the *Tyrannosaurus rex* is more related to the chicken than to the alligator or lizard. In other words, the birds are indeed survivor dinosaurs.

Actually, re-checking the fossil record, it has been found that many dinosaurs previously portrayed as lizards, had indeed a few or even a lot of feathers! Indeed, the external appearance of such dinosaurs was quite different (birds-like) than originally thought (lizards-like). The feathers can serve different purposes in the animal kingdom. For some dinosaurs, they may have played a role in the sexual courtship, but additionally they can be useful for flying and –most importantly– to maintain the corporal homeostasis. In fact, the feathers are a fantastic insulator. Thus, the penguins can survive the extremely low temperatures (–40 ºC) of the Antarctica that no other homeotherm can withstand. Precisely, the ~10-km wide impacting bolide that hit the Earth ~66 My, heated the planet initially, and the enormous amount of dust injected into the atmosphere blocked the sun light for years, which caused a drastic temperature drop of dozens of Celsius degrees, in a similar way as the predicted climatic effect of a hypothetical nuclear war (known as *nuclear winter*). The consequences were catastrophic for most species, which died due to the low temperatures and lack of food. Indeed, with no sunlight, the trophic chain breaks both in terrestrial and aquatic ecosystems, including the oceans: the photosynthetic plants and phytoplankton die or remain inactive (eg., seeds or spores), triggering a chain-reaction of deaths by hunger across the food pyramid, being the top and large super-predators the first to suffer such consequences, since they require a larger food supply to survive, being quickly extinct. Such asteroid or comet caused the extinction of 75% of the species at the time. Among the survivors were some feathers dinosaurs, which are the current birds (Dorado et al, 2010; Renne et al, 2013).

Cloning extinct species

The classical analytical equipment of the molecular biology laboratories typically requires hundreds of thousands or millions of molecules in order to detect them. The in-vivo molecular cloning can overcome such limitation, allowing to amplify any target nucleic acid. This approach was later on complemented and even substituted by the PCR amplification, which can be considered an in vitro cloning from the functional point of view. The new laboratory equipment can analyze single molecules in some

instances, as previously indicated, yet the cloning approaches are still needed and used in many cases.

The current and future technologies have the potential to bring extinct species "back to life" (Huynen et al, 2012; Kruger, 2013; Zimmer, 2013), sometimes incorrectly referred as "resurrecting" such species. The re-creation of a "Jurassic Park" (201 to 145 My) seems more remote if not impossible, since the DNA of such old age may be degraded (and even more in the case of the RNA). Nevertheless, ancient peptides from such samples may be recovered and sequenced, which opens new possibilities. Of course, the chances of cloning extinct species increase with the quality of the available biological sources, in which the age can be an important factor (in principle, the less older, the better), but the taphonomic and diagenetic history of the sample may be even more relevant, as previously described. There are three basic potential-scenarios to bring extinct species "back to life":

i) Synthetic genomics. The artificial synthesis of modern mammal mitochondrial (Gibson et al, 2010b) and bacterial genomes (Gibson et al, 2008a,b, 2010) open the possibility to create biological entities (Endy, 2008; Carr and Church, 2009; Cohan, 2010; Konig et al, 2010; Baker, 2011; Lynch and Gill, 2012; Ma et al, 2012; Maharbiz, 2012, Montague et al, 2012; Peisajovich, 2012; Cobb et al, 2013; Wang et al, 2013). The artificial synthesis of an ancient genome would be a much more difficult task, requiring a source of nucleic acids (peptides can also assist) that can be amplified, or at least sequenced and assembled into an in silico full genome that could be later on synthesized. There are certainly fossils of microorganisms, plants and animals, including bacteria in salt, organisms captured in amber, leaves, teeth, bones, eggs, etc (Vreeland et al, 2000; Martín-González et al, 2009; Oskam et al, 2010; Girard and Adl, 2011; Smejkal et al, 2011; Hofreiter et al, 2012; Schmidt et al, 2012) that might be used for such a goal. The available sequenced genomes and proteomes from related species could be also useful to assist the assemblies by mapping to reference genomes (Schubert et al, 2012), and even to fill the possible gaps, for which the knowledge of gene regulation, other "omics" (like metabolomics) and physiological networks can be also invaluable.

ii) Reverse-engineering current genomes to produce extinct ones. Such is the basis of the current project to generate a dinosaur by the modification of a chicken genome. This is not an easy task with the currently available knowledge and technology, and the information from related species and regulatory networks may be essential to create such *chickenosaurus* (Horner and Gorman, 2009).

iii) Cloning of ancient species from preserved tissues (somatic or germinal cells). A more pragmatic approach nowadays is to use preserved cells, and in particular their nuclei. Such is the case of the mammoth, which was extinct 4,000 y, because some well-preserved (frozen) specimens (including babies of such species) have been found in permafrost soils. The idea is to isolate a viable nucleus of such species, microinject it into a enucleated elephant ovule and implant it into a surrogate elephant mother. This approach is currently being considered and, if successful, it could recreate such species in a Pleistocene Park in Siberia (Fig. 4). There are also projects to restore the vanished ecosystems of the Oostvaardersplassen in the Netherlands and the Makauwahi Cave in Hawaii.

Figure 4. Woolly mammoth. Figure credit: Wooly Mammoth. © 2008 Royal British Columbia Museum <http://royalbcmuseum.bc.ca>, Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

Other projects involve the cloning of the moa (extinct 600 y) (Huynen et al, 2012), the European auroch (extinct in 1627), the dodo (extinct in 1662), the passenger pigeon (extinct in 1914), the Tasmanian marsupial wolf, also known as the Tasmanian tiger (because of its striped back) (extinct in 1936) (Pask et al, 2008), the Pyrenean ibex in Spain (extinct in 2000), etc.

On the other hand, we have previously reported the trials to amplify aDNA (Dorado et al, 2011) and in-vitro culture of seed tissues (Vásquez et al, 2011) from ancient samples (700 y) recovered from American burials in Peru. The maize breeding from the teosinte ancestors to the current hybrid varieties has taken 9,000 years, and it is expected that some ancient varieties may have desirable agronomical traits that may have been lost during the domestication of the species. Thus, it would be interesting to bring ancient maize varieties “back to life” and compare their genomes with those of the current varieties.

Cloning endangered species

There is also a general interest to restore previously extinct species that managed to survive elsewhere. Such is the case of the California condor (almost extinct in 1987, captured and reintroduced later on at different sites). Another species that due for restocking is the Guadalquivir river sturgeon in Spain (extinct in 1992), although

there is a debate whether it was the European sea sturgeon (*Acipenser sturio*) or the Adriatic sturgeon (*Acipenser naccarii*), which can be elucidated sequencing museum specimens to restock such river with its original species (Rowe et al 2011).

In the case of plants, the American chestnut is the subject of a recovery program after being almost extinct due to an Asian fungus infection. Of course, there is also the chance to find a living fossil, as was the case of the wrongly known as "Wollemi pine" (*Wollemia nobilis*), which in fact is not a pine at all, since it does not belong to the *Pinus* genus nor even the Pinaceae family, but instead is a coniferous tree belonging to the Araucariaceae family, being related to the *Araucaria* and *Agathis* genera. The Wollemi tree was considered extinct, with the oldest fossil dated 200 My. But, surprisingly, some living individuals of such plant were discovered by David Noble near Sydney, in the now known as Wollemi National Park (New South Wales, Australia). The species was named after his surname (*nobilis* for Noble), and subsequently propagated and world-wide distributed to prevent its extinction <http://www.wollemipine.com>.

Extinctions and evolution

The ecosystems can be destroyed as a consequence of internal (eg., geological-climatic) and external (eg., comet-climatic) events, as has happened many times in the biosphere of the Earth. Such extinction events may represent the annihilation of some, many and even most species at a particular time. But life on Earth has demonstrated that it is stubborn once spontaneously generated four Mdy ago, and somehow it has always escaped a total holocaust or annihilation, arising from the extinction event ashes and filing again the biosphere with new species.

This is because, paradoxically, the extinctions may also represent new opportunities for the evolution of some previously "minor" species. Indeed the current species of the planet Earth would be different if the planet had experienced a different number of mass-extinction events, mass-extinctions at different times or no mass-extinction events at all. In fact, the mass-extinction events albeit annihilating most of the existing species at a given time, also allow the regeneration similar or even completely new ones afterwards, given time. Thus, the mammals dominate the current biosphere with the human pinnacle because a massive extinction event wiped-out virtually all the dinosaurs. This catastrophic event gave the mammals the possibility to evolve and adapt to the new ecosystems, which otherwise would have been prevented by the dominating dinosaurs.

The humans can protect the biosphere and even bring extinct species "back to life" as previously described. Besides, the aDNA research can be also applied to assist the current wildlife conservation biology (Leonard, 2008; Drew, 2011; Lyman, 2012; Rick and Lockwood, 2013). The study of aDNA can be also useful to analyze old infections from their victim remains, to gather invaluable information to fight such diseases, like the leprosy by *Mycobacterium leprae* (Schuenemann et al, 2013), the tuberculosis by *Mycobacterium tuberculosis* (Donoghue et al, 2004; Djelouadji et al, 2011; Donoghue, 2011), the plague (Black Death) by *Yersinia pestis* (Bos et al, 2011), as well as any other diseases caused by different parasites (Zink et al, 2002; Dittmar, 2009).

At the same time, and paradoxically enough, the main danger for the current biosphere is the human species, besides the possible internal and external causes

previously described. Indeed, the humans can drastically alter the biosphere with a global nuclear war, contributing to the global warming and climate change, or just contaminating the environment. The best approach to avoid ecosystem disasters is to not interfere with them in an unsustainable way (eg., overexploitation, drastic modification or plain destruction), as well as to take care of them when required (eg., to extinguish massive fires, etc). If we want to survive, we should not destroy the Earth; instead we should protect our planet, which is the only known place harboring life in the universe. At the same time, we should keep on advancing our scientific and technological knowledge to protect ourselves and the biosphere from other internal or external menaces.

We must learn the dinosaur lesson: they arose ~231 My (Triassic) from the archosaurs. Interestingly, that was ~20 million years after the Permian–Triassic mass-extinction, which wiped out ~95% of the living species at the time. They were small bipedal predators like the *Eoraptor lunensis*, which gave them a strategic advantage to spot enemies and preys. So much, that they dominated the terrestrial ecosystems for 135 million years: from ~201 My (beginning of the Jurassic) to ~66 My (end of the Cretaceous). By comparison, the primates arose ~65 My. The aDNA data indicate that the humans and the great apes evolved ~4 to~ 8 My. The ancestor of all the modern humans arose in Africa ~200,000 y, showing a behavioral modernity ~50,000 y in what is known as the Upper Paleolithic Revolution or Great-Leap Forward. Such fascinating event involved cultural creativity and complex symbolic thought associated with the origin of the language. The agriculture Neolithic revolution started in the Fertile Crescent 15,000 y. The earliest recorded (written) history started ~6,000 y with the invention of writing.

To see it within a Cosmic Calendar perspective, if the 15 Mdy age of the universe are reduced to a year, the Big Bang would have taken place on the beginning of the 1st of January, whereas the modern humans would have arisen at 23:52 h of the 31st of December of such year <http://en.wikipedia.org/wiki/Cosmic_Calendar>. On the other hand, if the five Mdy since the formation of the Earth are reduced to 24 h, then we have been living just one minute and 17 seconds <http://www.geology.wisc.edu/homepages/g100s2/public_html/history_of_life.htm>. So, we are but a sigh (only eight seconds) in the universe evolution.

Concluding remarks

The current nucleic-acid and peptide sequencing technologies allows to analyze ancient remains with an unprecedented resolution power. For the first time, ancient genomes have been sequenced. Additionally, the fact that peptides may remain when nucleic acids have been degraded opens the door to studies of older samples. Thus, these developments have demonstrated that the current humans, Neandertals and Denisovans belong to the same species, and that the birds are surviving dinosaurs. It could be even possible to analyze ancient transcriptomes with the third-generation sequencing platforms. Finally, and most exciting, is the prospect of bringing extinct species “back to life”, which is certainly a challenging goal.

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