Bioarchaeology to bring back scents from extinct plants - Review

Gabriel Dorado [1], Fernando Luque [2], Plácido Pascual [3], Inmaculada Jiménez [4], Francisco Javier S. Sánchez-Cañete [5], Patricia Raya [6], Jesús Sáiz [7], Adela Sánchez [7], Teresa E. Rosales [8], Víctor F. Vásquez [8], Pilar Hernández [9]

[1] Author for correspondence, Dep. Bioquímica y Biología Molecular, Campus Rabanales C6-1-E17, Campus de Excelencia Internacional Agroalimentario (ceiA3), Universidad de Córdoba, 14071 Córdoba (Spain), eMail: <bb1dopeg@uco.es>; [2] Laboratorio de Producción y Sanidad Animal de Córdoba, Ctra. Madrid-Cádiz km 395, 14071 Córdoba; [3] Laboratorio Agroalimentario de Córdoba, Consejería de Agricultura y Pesca, Junta de Andalucía, 14004 Córdoba; [4] IES Puertas del Campo, Avda. San Juan de Dios 1, 51001 Ceuta; [5] EE.PP. Sagrada Familia de Baena, Avda. Padre Villoslada 22, 14850 Baena (Córdoba); [6] Dep. Radiología y Medicina Física, Unidad de Física Médica, Facultad de Medicina, Avda. Menéndez Pidal s/n, Universidad de Córdoba, 14071 Córdoba; [7] Dep. Farmacología, Toxicología y Medicina Legal y Forense, Facultad de Medicina, Avda. Menéndez Pidal, s/n, Universidad de Córdoba, 14071 Córdoba; [8] Centro de Investigaciones Arqueobiológicas y Paleoecológicas Andinas Arqueobios, Apartado Postal 595, Trujillo (Peru); [9] Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Alameda del Obispo s/n, 14080 Córdoba.

Abstract

The union of classical archaeology with biology, in general, and molecular biology, in particular, is reaching goals that were considered impossible just a few years ago. That has been possible thanks to the development of technologies like first-, second- and third-generation sequencing of nucleic acids. Among bioarchaeology challenges is de-extinction of ancient species. That represents huge technological challenges. Ethical issues and excessive costs should be also taken into consideration. So far, it has been possible to clone ancient genes, like the ones encoding scents from extinct plants. They include Wynberg conebush (*Leucadendron grandiflorum*), Maui’s mountain-hibiscus (*Hibiscadelphus wilderianus*) and Falls-of-the-Ohio scurfpea (*Orbexilum stipulatum*). Thus, synthetic biology has been exploited to clone genes encoding enzymes catalyzing biosynthesis of scent molecules, like terpenes. They have been expressed in yeasts, producing ancient fragrances. This is just an exciting example of the potential of this technology.

Key words: revivalism, resurrection, ancient DNA, aDNA, bioinformatics, Jurassic Park.

Resumen

La unión de la arqueología clásica con la biología, en general, y la biología molecular, en particular, está alcanzando objetivos que eran considerados imposible hace tan solo unos años. Ello ha sido posible gracias a el desarrollo de tecnologías como la secuenciación de ácidos nucleicos de primera, segunda y tercera generación. Entre los retos de la bioarqueología se encuentra la des-extinción de especies antiguas. Ello representa grandes retos tecnológicos. Los aspectos éticos y costes excesivos también deben tenerse en cuenta. Hasta el momento, ha sido posible clonar genes antiguos, como los que codifican aromas de plantas extintas. Estas incluyen el arbusto de piña Wynberg (*Leucadendron grandiflorum*), el hibisco de montaña de Maui (*Hibiscadelphus wilderianus*) y el guisante escamoso de las Cascadas de Ohio (*Orbexilum stipulatum*). De este modo, la biología sintética ha sido explotada para clonar genes codificantes de enzimas que catalizan la biosíntesis de moléculas aromáticas, como los terpenos. Han sido expresados en levaduras, produciendo fragancias antiguas. Esto es, nada más y nada menos que un ejemplo excitante del potencial de esta tecnología.

Palabras clave: avivamiento, resurrección, ADN antiguo, ADNa, bioinformática, Parque Jurásico.

Introduction

Archaeology studies ancient remains and bioarchaeology the ones related to biological entities. Initially, those remains could be studied using morphological and analytical approaches, including anatomical, isotopic, mathematical and statistical ones. Fortunately, technological breakthroughs in molecular biology have allowed quantum leaps in this fascinating research topic (Linderholm, 2016). Thus, the development of first-generation sequencing (FGS) allowed to read ancient DNA (aDNA) for the first time. That was accomplished using a traditional molecular cloning approach. This way, aDNA was isolated and ligated into λ gt10 vector. Such recombinant DNA was used to transform *Escherichia coli* cells, effectively amplifying it in vivo (Higuchi et al, 1984). Yet, that is a tedious and time-consuming methodology. A much faster and convenient in vitro DNA amplification was first described with exquisite detail later on (Kleppe et al, 1971; Panet and Khorana, 1974). Nevertheless, they considered it not viable, due to methodological limitations at the time. Fortunately, the same methodology was reinvented and popularized 14 years later, with the name of polymerase chain-reaction (PCR). It was erroneously considered as a low-relevance “methods paper” and rejected by *Nature* journal, being eventually published in *Science* magazine (Saiki et al, 1985). Such technology allowed to amplify and sequence aDNA in a quick and convenient way, without requiring previous in vivo molecular cloning approaches. Additionally, second-generation sequencing (SGS) increased throughput and reduced overall pricing, allowing to sequence ancient genomes for the first time. That included the Neanderthal one (Green et al, 2010). Third-generation sequencing (TGS) allows to sequence single-molecules, without requiring previous amplification. Such revolution has been applied to study ancient DNA, like the one isolated from a Pleistocene horse bone (Orlando et al, 2011), and could be potentially used to sequence even ancient RNA (aRNA) (Dorado et al, 2007-2018).

One of the challenges in bioarchaeology is the so-called de-extinction (sometimes called revivalism or resurrection) of extinct species. That is a highly complex task, involving huge technological challenges (O’Connor, 2015; Campagna et al, 2017; Corlett, 2017; Dorado et al, 2017; Iacona et al, 2017; McCauley et al, 2017; Robert et al, 2017; Shapiro, 2017; Steeves et al, 2017). It also has legal and ethical implications in some instances, including environmental risks like loss of biodiversity and animal welfare issues, besides excessive costs (IUCN/SSC, 2013; Seddon et al, 2014; Bennett et al, 2017; Wagner et al, 2017; Tanentzap and Smith, 2018). There are different steps and approaches in such general topic of bringing back to life something from the past. That includes cloning and expressing ancient genes (Thornton, 2004; Garcia and Kacar, 2019), like the ones encoding scents or fragrances from extinct plants (Jacobsen, 2019).

Cloning approaches

Traditional laboratory equipment requires many molecules in order to analyze them. There are several cloning approaches to reach the goal of molecule production. The first one is known as molecular cloning, as described above. Typically, it involves generating competent prokaryotic cells, like the ones of *Escherichia coli* or *Lactobacillus* spp bacteria. Eukaryotic cells can also be transformed, like *Pichia pastoris* and brewer’s yeast (*Saccharomyces cerevisiae*). Furthermore, plants can be regenerated from a single transformed cell using *Agrobacterium* spp. When the plant species cannot be transformed that way, other methodologies involving electroporation and bioballistic o biolistic (“gene gun”) methods can be used. In the case of animals, DNA fragments can be injected into cells to transform them. Somatic cells can be immortalized and somatic stem cells can be differentiated. Germinal cells, like ovules, can produce a complete organism. These in vivo cloning strategies have the added advantage of allowing characterization of gene expression. Curiously, they were greatly improved and sometimes even replaced with in vitro methodologies, like PCR and isothermal amplification. They reduced effort, time, and cost to produce large quantity of required molecules. This way, it is now possible to easily amplify DNA fragments, including desired genes of ancient remains, provided that nucleic acids with enough quality are isolated from them. Indeed, the threshold detection is one molecule for in vitro amplification methodologies.

Extinct plant species with potential scent interest

Extinct plant species with potential fragrance interest include Wynberg conebush (*Leucadendron grandiflorum*) from South Africa, as well as two from the United States of America (USA): Maui’s mountain-hibiscus (*Hibiscadelphus wilderianus*) from Hawaii; and Falls-of-the-Ohio scurfpea or largestipule leather-root from Indiana (*Orbexilum stipulatum*). Wynberg-conebush was extinct in 1806 (Fig 1).

Figure 1. **Wynberg conebush.** © 2019 tonyrebelo, iNaturalist Network <https://www.inaturalist.org>, Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

On the other hand, Maui's mountain-hibiscus became extinct later on, in 1912 (Fig 2).

Figure 2. **Maui's mountain-hibiscus**. © 2019 Smithsonian Institution, National Museum of Natural History, Department of Botany <https://collections.nmnh.si.edu/search/botany>, iNaturalist Network <https://www.inaturalist.org>, Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

Finally, Falls-of-the-Ohio scurfpea was a legume endemic of a few rocky islets of the Ohio river, which were drowned by dams in the 1920 decade (Fig 3).

Figure 3. **Falls-of-the-Ohio scurfpea**. © 2019 Charles Wilkins Short (The Philadelphia Herbarium at the Academy of Natural Sciences <http://ph.ansp.org>), Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

Cloning, expression and characterization of scents from extinct plants

Synthetic biology can be exploited to bring back fragrances from extinct plants. Ancient specimens are usually precious and scarce. Thus, only small amounts of them are usually available for the destructive process of DNA isolation. Fortunately, current methodologies allow to sequence tiny amounts of DNA. This way, it is possible to identify genes encoding enzymes catalyzing biosynthesis of scent molecules, including terpenes (Priya et al, 2018), like sesquiterpene synthases (SQS). Yet, ancient DNA is usually degraded, so only short stretches of DNA may be generated. Lucky, some of such genes from some living species have been already sequenced. They can be used as reference to assemble reads from ancient DNA sequencing, generating the corresponding contigs through bioinformatics workflows.

That should allow to generate complete gene sequences, without gaps or ambiguities (in the best scenario). Unfortunately, that is not usually expected when sequencing aDNA, due to both physical and chemical degradation of nucleic acids (Allentoft et al, 2012; Dorado et al, 2013; Linderholm, 2016). Therefore, reference genes should be used to overcome these hurdles. Reconstructed ancient genes are then chemically generated with traditional DNA synthesizers (based on chemical reactions), or the new revolutionary DNA printers [like the ones based on biochemical reactions, using terminal deoxynucleotidyl transferase (TdT) enzymes], and ligated into expression vectors. The latter can be plasmids, that are used to transform competent prokaryotic or eukaryotic cells, as shown above. Transformed cells can grow and express cloned genes, biosynthesizing scent molecules. They can be identified with different analytical methods, like gas chromatography coupled with mass spectrometry (GC-MS) (Begnaud and Chaintreau, 2016) (Fig. 4).

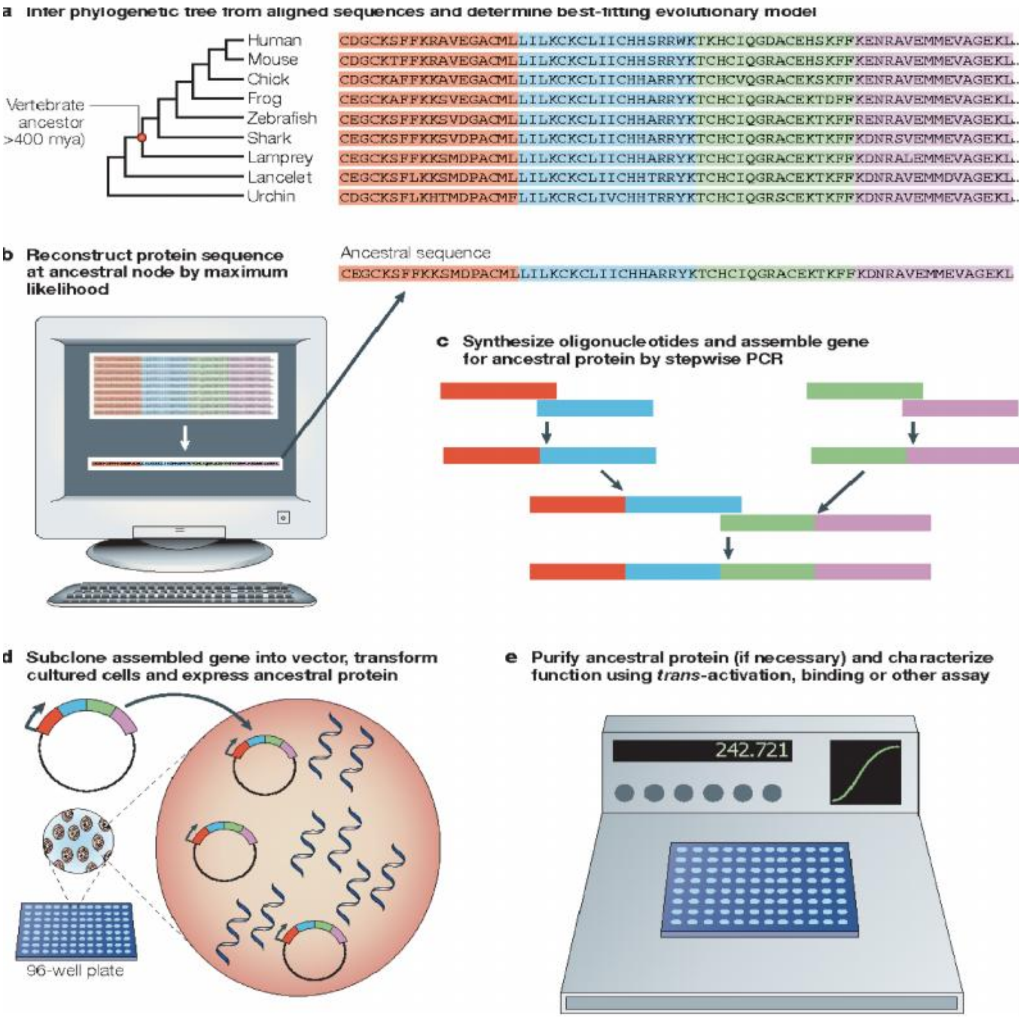

Figure 4. **Cloning ancient genes.** If possible, ancient DNA is sequenced. If required, ancient genes are reconstructed using reference ones from living species. Chosen genes are synthesized and cloned into microorganisms, which express them, producing ancient proteins. © 2019 Springer Nature Publishing (Thornton, 2004). See also specific example of cloning ancient scent genes at <https://static.scientificamerican.com/sciam/assets/Image/2019/saw0219Jaco31_d.png> (Jacobsen, 2019).

Concluding remarks and future prospects

Bringing back fragrances of extinct plants is just one example of the power of bioarchaeology, linking archaeology with biology, in general, and molecular biology, in particular. Other examples may involve diverse genes of interest. Additionally, the prospect of sequencing aRNA (including ancient transcriptomes) using TGS is provocative and exciting. Most challenging will be bringing to life extinct species. Work is currently being carried out to accomplish such a goal with some species (Dorado et al, 2017). They include the passenger pigeon (*Ectopistes migratorius*) and the woolly mammoth (*Mammuthus primigenius*). Yet such possibility has raised concerns and questions, as pointed out in the "Guidelines for Reintroductions and Other Conservation Translocations", being published by the International Union for the Conservation of Nature (IUCN) <https://www.iucn.org> and Species Survival Commission (SSC) <https://iucn-ctsg.org>. For instance, de-extinction can be considered an ecological enrichment involving conservation translocation. Therefore, risks associated with species reintroduction must be taken into account, in order to prevent unsuitable reintroduced candidates (IUCN/SSC, 2013; Seddon et al, 2014; Adams, 2017; Sandler, 2017a, b; Wagner et al, 2017; Browning, 2018; Novak, 2018; Tanentzap and Smith, 2018). Indeed, the ecosystem in which such species became extinct (for instance, Siberia with woolly mammoths, thousands of years ago) may be quite different from the current one, with different environmental conditions and species. Putative dangers of de-extinctions were popularized with the *Jurassic Park* science-fiction novel (Crichton, 1990), adapted by a film franchise (Spielberg, 1993-2021), in relation to dinosaurs and other reptiles. Today we know that such a goal is science fiction, since such old nucleic acids are completely degraded beyond recovery (Allentoft et al, 2012; Dorado et al, 2013; Linderholm, 2016). But it highlights this topic in an entertaining way, and thus has captured people's imagination for decades.

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