Genetic Time Travel

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ABSTRACT At its core, genetics is a historical discipline. Mutations are passed on from generation to generation and accumulate as a result of chance as well as of selection within and between populations and species. However, until recently, geneticists were confined to the study of present-day genetic variation and could only indirectly make inferences about the historical processes that resulted in the variation in present-day gene pools. This "time trap" has now been overcome thanks to the ability to analyze DNA extracted from ancient remains, and this is about to revolutionize several aspects of genetics.

KEYWORDS ancient DNA; evolution

OVER 30 years ago, the first evidence that DNA could survive in dead organisms emerged (Higuchi *et al.* 1984; Pääbo 1984). With the invention of the polymerase chain reaction (PCR) it became possible to reproducibly retrieve old DNA sequences (Pääbo *et al.* 1989). This resulted in novel insights about the relationships of extinct animals and in the determination of the first DNA sequences from a Neandertal, the closest extinct relative of present-day humans (Krings *et al.* 1997). However, early enthusiasm was tempered by the realization that contamination with present-day human DNA made studies of ancient human remains challenging (Pääbo *et al.* 2004). Whereas the retrieval of multicopy DNA sequences such as mitochondrial DNA was often possible, the study of single-copy nuclear DNA from diploid organisms was fraught with difficulty (for a popular account, see Pääbo 2014b). This situation changed with the advent of high-throughput DNA sequencing technologies at the beginning of this millennium.

High-throughput DNA sequencing circumvents the need to directly apply the PCR to DNA extracts prepared from ancient tissues. Instead, the extracts are used to produce DNA libraries that can then be sequenced or used to isolate DNA fragments of interest by hybridization capture (Burbano *et al.* 2010). This has several advantages. First, the vast majority of the DNA molecules preserved in ancient tissues are degraded to lengths <50 bp (Green *et al.* 2009). Such short molecules can be studied by sequencing molecules in DNA libraries, whereas the application of PCR requires that a large proportion of the preserved molecules are used for priming sites, thus making the study of molecules shorter than $\sim$40 bp impossible. Second, while the PCR by necessity focuses on longer molecules, the high-throughput sequencing techniques make it possible to focus on the older and shorter molecules, avoiding contaminating DNA molecules that often are longer (Krause *et al.* 2010a). Third, the chemical modifications that accumulate in ancient DNA over time cause characteristic miscoding lesions that accumulate at the ends of DNA molecules (Sawyer *et al.* 2012). Although inaccessible to direct PCR, these substitution patterns can be detected using high-throughput sequencing. Because they are largely absent in modern DNA, they can be used to authenticate ancient DNA, and when present-day, contaminating DNA is present in an extract the analysis can be confined to molecules that carry such substitutions and thus are likely to be ancient (Krause *et al.* 2010a).

Refinements of the techniques that allow short DNA sequences to be extracted efficiently (Dabney *et al.* 2013) and libraries to be made from small amounts of damaged DNA (Meyer *et al.* 2012), as well as the finding that DNA is particularly likely to survive in the petrous part of the temporal bones of humans and animals (Gamba *et al.* 2014), have made it possible to retrieve genome-wide DNA data from large numbers of remains and from warmer areas of the world where preservation is less optimal (Gallego Llorente *et al.* 2015). The ability to go back in time will revolutionize genetics in at least four areas.

Extinct Organisms

Most species that have existed on the planet are extinct. Remains of some of these are preserved as fossils, and from

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a fraction of these DNA can now be retrieved. This contributes to our ability to understand evolutionary processes. For example, the elucidation of the phylogeny of flightless ratite birds such as moas, kiwis, and ostriches, most of which are extinct, has shown that flightlessness has evolved multiple times in the Southern Hemisphere (Cooper *et al.* 1992). Other examples of convergent evolution have been found among extinct marsupial carnivores in Australia and South America (Thomas *et al.* 1989), and DNA from many extinct animals such as cave bears (Hänni *et al.* 1994), giant ground sloth (Höss *et al.* 1996), and the saber-toothed cat (Janczewski *et al.* 1992), to name a few, have been retrieved. Whole genomes from the mammoth (Lynch *et al.* 2015; Palkopoulou *et al.* 2015) and a 700,000-year-old horse (Orlando *et al.* 2013) presage the complete genomes of many extinct species that will be sequenced over the next decade. This will clarify the evolutionary relationships of these species and open the possibility of understanding functional adaptations in extinct organisms.

Human Origins

Genome sequences of Neandertals (Green *et al.* 2010; Prüfer *et al.* 2014) provide a unique perspective on human origins by showing that Neandertals contributed to the gene pool of present-day humans. Our ancestors also include a distant Asian relative of Neandertals, the Denisovans, which was discovered using DNA from a small finger bone found in the Russian part of the Altai Mountains (Krause *et al.* 2010b; Reich *et al.* 2010). There is emerging evidence that such genetic contributions may have substantial physiological effects in present-day humans, for example, for the immune system (Abi-Rached *et al.* 2011), for lipid metabolism (Khrameeva *et al.* 2014), and for adaptation to high altitude (Huerta-Sanchez *et al.* 2014). Neandertal genetic variants also have medical consequences, for example, with respect to type 2 diabetes (Consortium *et al.* 2014), *Helicobacter pylori* susceptibility (Dannemann *et al.* 2016), and depression, actinic keratosis, hypercoagulation, and other diseases (Simonti *et al.* 2016).

The availability of the Neandertal and Denisovan genome sequences also allows us to identify the genetic changes shared by all (or almost all) present-day humans, but absent in Neandertals and the great apes. Their total number is only ~30,000. An important undertaking over the next few years will be to understand the functional consequences of these changes, which are likely to include some that underlie unique aspects of the modern human phenotype (Pääbo 2014a). This not only may illuminate the biological basis for human evolutionary history over the past 100,000 years, but also may make inroads into the understanding of diseases such as autism that may affect aspects of cognition that are unique to humans.

Human History

The ability to study genetic variation in past human populations will revolutionize archaeology by providing direct insights into migrations and population turnover in the past. Genome-wide analyses of hundreds of West Eurasians spanning the past 10,000 years provide direct evidence for at least two major human migrations into Europe at the beginning and the end of the Neolithic (Haak *et al.* 2015). A recent study on Pleistocene human remains across Europe provided genetic evidence for a previously unknown population turnover in Europe at the end of the last Ice Age, ~14,000 years ago, highlighting how genetic studies can reveal unsuspected events in human history (Posth *et al.* 2016). Ancient and present-day genomic data will also provide new perspectives on historically documented events such as the Anglo-Saxon invasion of Great Britain by allowing estimates of the number of migrants and the reconstruction of admixture patterns (Schiffels *et al.* 2016).

Now that large numbers of ancient individuals can be efficiently analyzed, genetic transects through space and time will allow the spread of alleles to be monitored over time, allowing biological adaptations to be correlated with environmental or cultural change and with epidemics (Mathieson *et al.* 2015). In fact, genetic studies of human remains recovered at excavations are likely soon becoming a standard tool in archaeology similar to radiocarbon dating, allowing genetics to enter into a fruitful symbiotic relationship with archaeology.

Evolution of Pathogens

Despite tremendous progress in biomedical research, infectious diseases still pose a major threat to human health. However, surprisingly little is known about the evolution and historical dissemination of most human pathogens. Even the etiological agents of past pandemics and the zoonotic origin of human diseases are often subjects of speculation. The reconstruction of pathogen genomes from ancient skeletal remains allows evidence-based diagnosis of past diseases and the direct study of the evolutionary history of pathogens and host–pathogen interactions. For example, the recent reconstruction of ancient *Yersinia pestis* genomes provided unequivocal evidence for the presence of bubonic plague during the Medieval Black Death pandemic in Europe and demonstrated that no major changes in the genetic makeup of this pathogen have occurred during the past 660 years (Bos *et al.* 2011). *Mycobacterium tuberculosis* genomes from 1000-year-old human remains exhumed on the coast of Peru were found to be most closely related to strains causing tuberculosis in seals and sea lions, suggesting an unexpected zoonotic origin of tuberculosis in the New World (Bos *et al.* 2014). Moreover, the study of immune genes in individuals who succumbed and who survived pandemics in the past will reveal how pathogens and the immune system interact and may provide new strategies for prevention and treatment of infectious diseases.

Conclusions

During just the past year, hundreds of genome-wide data sets from ancient humans have been published (Allentoft *et al.*

2015; Haak *et al.* 2015; Mathieson *et al.* 2015). This development will undoubtedly accelerate and extend to other organisms. However, DNA retrieval also has limitations. Because ancient DNA is almost invariably degraded to short fragments, *de novo* assemblies of genomes will remain difficult or impossible, and mapping to repetitive parts of the genome will be only partially possible. DNA preservation is also limited by age and by environmental conditions. Outside the permafrost, the oldest hominin DNA retrieved to date is a little over 400,000 years old (Meyer *et al.* 2014, 2016). In most parts of the world, it will probably not be possible to study DNA sequences much older than half a million or at the most a million years. But genetic studies of humans and other organisms as well as their pathogens and commensals across the past 100,000 years will soon become routine.

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