

**Title:** Migrating Microbes: what pathogens can tell us about population movements and human evolution

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**Abstract**

**Background:** The biology of human migration can be observed from the co-evolutionary relationship with infectious diseases. While many pathogens are brief, unpleasant visitors to human bodies, others have the ability to become life-long human passengers. The story of a pathogen's genetic code may, therefore, provide insight into the history of its human host. The evolution and distribution of disease in Africa is of particular interest, because of the deep history of human evolution in Africa, the presence of a variety of non-human primates, and tropical reservoirs of emerging infectious diseases.

**Methods:** This study explores which pathogens leave traces in the archaeological record, and whether there are realistic prospects that these pathogens can be recovered from sub-Saharan African archaeological contexts.

**Results:** Three stories are then presented of germs on a journey. The first is the story of HIV's spread on the back of colonialism and the railway networks over the last 150 years. The second involves the spread of *Schistosoma mansoni*, a parasite which shares its history with the trans-Atlantic slave trade and the origins of fresh-water fishing. Finally, we discuss the tantalising hints of hominin migration and interaction found in the genome of human herpes simplex virus 2.

**Conclusions:** Evidence from modern African pathogen genomes can provide data on human behaviour and migration in deep time and contribute to the improvement of human quality-of-life and longevity.

**Keywords:** Archaeology, migration, ancient DNA, pathogens, microbial archaeology, human evolution, sub Saharan Africa

## Introduction

There is significant evidence that human populations currently suffer, and may have suffered for millions of years, from infectious diseases shared with or closely related to the infectious diseases of wild primates (Wolfe et al. 2007). These conclusions are drawn largely by studying the phylogenetic relationships of extant pathogens, both those that are exclusively human pathogens, and those we share with other primates. This has led researchers to conclude that many infectious diseases have been co-evolving with humans and our ancestors for millennia (Houldcroft & Underdown 2016). Reviews by (Wolfe et al. 2007; Trueba & Dunthorn 2012; Harkins & Stone 2015; Houldcroft & Underdown 2016) highlight the gaps in our understanding of the origins of diseases, and especially their relationship with human evolution, behaviour and migration in Africa. Besides being the cradle of behaviourally modern *Homo sapiens* (Mourre et al. 2010; Henshilwood et al. 2011; Henshilwood et al. 2009; D'Errico et al. 2012) sub-Saharan Africa also brings together exceptionally rich biodiversity with pathogen abundance (Just et al. 2014). Prehistoric sub-Saharan African populations who inhabited the region over the past 150 000 years are therefore believed to characterise the human ancient disease landscape.

The first modern human dispersals occurred within Africa during MIS 5 (Marine Isotope Stage 5) some 135 000 to 75 000 years ago (ka). The increasing aridity experienced during MIS 5 likely played a role in the expansion of human populations in central and eastern Africa, ultimately triggering the dispersal of humans out of Africa after c. 65 ka. The development of modernity in early human populations has been linked to various phases of technological and behavioural innovation. While the triggers for these sporadic pulses of technological innovation are not obvious, the incidence of innovations appears to be linked to instances of abrupt climate change (Ziegler et al. 2013). When rainforests expanded during MIS 5, hunters of grassland species moved north and south, taking bifacial technology to North Africa (the Aterian), and South Africa (the Still Bay) (Wadley 2007). Thus, and by the beginning of MIS 5, two behaviourally fully modern human populations were isolated at the opposite ends of Africa (Rito et al. 2013). One thrived on the southern coastal plain in South Africa after 145 ka (Marean et al. 2007; Wadley 2007; Henshilwood et al. 2009; Henshilwood et al. 2011) and the other prospered in the Maghreb, North Africa after 140 ka (Osborne et al. 2008; Barton et al. 2009; Castañeda et al. 2009; Garcea 2012). It is from these isolated populations that the earliest archaeological indications of 'fully modern' and symbolic human behaviour derive. While the regional distributions of projectile point styles may indicate the existence of complex social networks, the first cultural traditions emerge just before 100 ka, as shown by the engraved ochres from Blombos Cave (Henshilwood et al. 2009), Klein

Kliphuis Rock Shelter (Mackay & Welz 2008), Pinnacle Point Cave 13B (Watts 2010) and Klasies River Cave (D'Errico et al. 2012). From 92 ka to 72 ka, evidence for personal ornamentation, in the form of perforated marine shell beads, appears for the first time during the Still Bay and the Aterian (Bouzouggar et al. 2007; Bar-Yosef Mayer et al. 2009; Zilhão et al. 2010).

While much is known about the evolution of human technological competence and symbolic capacity, the influence that diseases had on the biological and social evolution of our species is an essential and often overlooked aspect of our developmental history. Disease certainly appears to have played a significant role in the evolution and geographic distribution of ancient behaviourally-modern Africans. In fact, the scarcity of evidence for human occupation in the tropical regions of central and West Africa (Webb 2005) has been attributed to disease, specifically malaria (Spriggs 2008). Malaria infection occurs when female mosquitoes inject saliva containing plasmodial sporozoites (parasites) into the host during feeding (Tolle 2009). Of the roughly 250 *Plasmodium* species, *P. vivax*, *P. malariae*, *P. falciparum* and *P. ovale* are highly anthropophilic (Ollomo et al. 2009). *P. falciparum* is closely related to *P. reichenowi* and possibly originated from parasites specific to chimpanzees (Rich et al. 2009) and bonobos (Krief et al. 2010) some 3 million years ago (mya), although, data from faecal sampling suggests that gorillas were the likely host species for *P. falciparum*, before a cross-species transmission event to humans or our ancestors (Liu et al. 2010). *P. malariae* diverged from a parasite of chimps, or both chimps and hominins, around 3.5 mya (Rutledge et al. 2017). The presence of malaria in sub-Saharan Africa therefore predates the emergence of anatomically modern humans 200 ka (White et al. 2003; McDougall et al. 2005), and mitochondrial mtDNA analyses confirm that early forms of *P. falciparum* were present by at least 100 ka (Silva et al. 2011). The parasite subsequently spread from Africa to the Near East and Asia between 90 ka and 80 ka, and to Europe after 40 ka (Tanabe et al. 2010). These ages are consistent with current hypotheses concerning the spread of *Homo sapiens* (Armitage et al. 2011). *Plasmodium vivax*, which today is principally a pathogen of Asia and Latin America, evolved in chimpanzees or gorillas in central Africa. *P. vivax* then radiated across the world, most likely as the result of human migration (Liu et al. 2010).

For humans, the avoidance of ecological niches conducive to mosquitoes presents an obvious means to prevent the risk of malaria infection, and also other mosquito-borne diseases such as dengue fever, West Nile virus, Chikungunya and yellow fever. In Namibia and Botswana, Singer (Singer 1960) observed that San hunter-gatherers who lived more than 25 km from water sources were not susceptible to

mosquito-borne diseases. Besides ambient temperature and precipitation (Tonnang et al. 2010), proximity to water plays an important role in the prevalence of malaria. Singer (1960) therefore suggested that Kalahari San migratory routes were deliberately structured to avoid waterlogged areas during summer. There is no conclusive evidence for the incidence of the HbS, b-thalassemia or G6PD traits amongst the Kalahari San (Jenkins et al. 1968; Tishkoff et al. 2001; Kwiatkowski 2005), suggesting that malaria did not exert selective pressure on these groups. Similarly, prehistoric humans may simply have circumvented areas prone to seasonal malaria transmission (including the tropical region of Central Africa), and in this regard the absence of the HbS sickle-cell, b-thalassemia and the G6PD traits amongst the Kalahari San is significant. In addition, whereas the Duffy negative blood group locus is widespread amongst sub-Saharan populations, both the FY\*A and FY\*B antigens are rare amongst the San of the Kalahari Desert (Howes et al. 2011). This seems confirm the notion that ecological niche avoidance restricted the susceptibility of humans to mosquito-borne diseases (Singer 1960; Dugassa et al. 2009; Wadley 2012).

### **The relevance of ancient African diseases for modern human society**

Resembling our co-evolutionary history with malaria parasites, gaining information about the incidence of disease in prehistoric Africa is important as tropical pathogens and parasites had, and still exert, a significant impact on the evolution of our species. Our rise to being the predominant species on earth is the result of complex interactions between biological and cultural processes, and during the initial stages of our cognitive, technological and cultural evolutionary history, all these processes occurred in sub-Saharan Africa. Current epidemiologic transition models tend to associate the emergence of most human diseases with changes in living conditions associated with agricultural innovation and higher population densities during the Neolithic Period, c. 12 ka (Omran 1971). As a result, the search for the origins of diseases has focussed primarily on domestic animals and environments outside Africa. But, many of these tropical infections are likely to have played a role in the human evolutionary process for much lengthier periods of time (Barrett et al. 1998).

Of the approximately 2100 species of microorganisms that interact directly with humans (Wardeh et al. 2015), 1415 species are known to be pathogenic, including 217 viruses and prions, 538 bacteria, 307 fungi, 66 protozoa and 287 helminths (Taylor et al. 2001; Woolhouse & Gowtage-Sequeria 2005). Approximately 65% of these are zoonotic (Lloyd-Smith et al. 2009) and 177 (8.4%) cause emerging

infectious diseases (Dutour 2013). Of these, at least 20 have certain to probable African origin, including hepatitis B, measles, cholera, dengue fever, *P. falciparum* malaria and leishmaniasis, plague and smallpox (Wolfe et al. 2007; Houldcroft & Underdown 2016). The potential impact of disease on prehistoric humans is illustrated by the fact that ~60% of modern hunter-gatherers succumb to disease before reaching reproductive age (c. 15 years) (Gurven & Kaplan 2007).

But how are we affected by disease today? And what can we learn from the study of ancient human disease pathogens? The current global disease burden is dominated by both ancestral (Wolfe et al. 2007; Houldcroft & Underdown 2016) and novel emerging or infectious diseases (Langwig et al. 2015; Plummer et al. 2016). Pathogens result in nearly 11 million human deaths per annum and are responsible for 51% of years of life lost globally (Dunn et al. 2010). Research concerning ancient pathogens can contribute significantly to our understanding of infectious disease evolution in a number of ways. These include improving our understanding of when (and how) virulence evolves in pathogens, when certain pathogens (or parasites) became human pathogens and even whom to prioritise during vaccination campaigns. Studying ancient pathogen DNA (apDNA) is therefore not just of interest to archaeologists, but is also of relevance to public health researchers and molecular biologists.

Because our temporal frame of reference is restricted, and since changes in disease aetiology (including virulence and communicability) frequently occur over longer time periods (Achtman 2016), we do not fully comprehend the processes implicated in disease evolution and emergence. Comparative genomics can be used to reconstruct short-term evolutionary histories of pathogen clades whose diversity converges towards a 'most recent common ancestor' (MRCA) that existed decades or even millennia ago (Der Sarkissian et al. 2015). Genetic changes can be observed in the genomes of bacteria, viruses and parasites and occur through single nucleotide mutations, insertions or deletions or genomic rearrangements. Since mutations play an important role in pathogen evolution and virulence, information derived from apDNA sequences have incredible epidemiological potential. Prehistoric pathogen research can therefore contribute to our understanding of infectious disease evolution by providing chronologically-secure (dated) sequence data to integrate into phylogenetic reconstructions. For a number of reasons, studies of extant pathogen genomes and estimations of the age of a pathogen based on genetic data represent only minimal estimates of the age of a taxon (Achtman 2016). But by anchoring pathogen emergence dates and mutation rates, apDNA research can provide crucial calibration points to estimate the timing of divergence events (DeWitte et al. 2016).

For example, the sequencing of the 1918-1919 Spanish influenza (H1N1) virus genome yielded new insight into virus biology and pathogenesis ([Taubenberger et al. 2012](#)). It is believed that H1N1 emerged in 1893 and that, by 1918, the virus had already accumulated ~375 mutations, at a rate of approximately 15 mutations per year! One of these involved the acquisition of mutations derived from the H5N1 avian virus, and the result was the 1918 Spanish influenza pandemic. Subsequent human-to-human transmission barriers were crossed by the novel zoonotic influenza virus, finally triggering the 1918 pandemic. This illustrates the devastating consequences of influenza virus cross-species transmission ([Reperant et al. 2012](#)). The ensuing pandemic viruses of 1957, 1968, and 2009 all descended from the original 1918 virus. The reconstruction of the 1918 virus facilitated the rapid assessment of the potential virulence of the 2009 H1N1 pandemic virus ([Medina et al. 2010](#)). The 1918 Spanish influenza virus-specific B cell clones could still be recovered from elderly survivors 90 years after their exposure to the virus but before their exposure to the 2009 pandemic virus ([Taubenberger et al. 2012](#)). This realisation provided a scientific rationale for targeting the initial 2009 H1N1 pandemic vaccine to those who needed it most, namely younger persons who had not been exposed to the cross-protective 1918 virus or to its seasonally prevalent descendants. Thus, early in the 2009 pandemic, limited vaccine supplies that might have been misdirected to the traditional (elderly) risk group was administered to younger persons, who benefitted most.

Studying the evolution of infectious diseases, through modern and apDNA, has implications for chronic disease too. There are a number of cancers that are partly or wholly attributable to oncogenic viruses and bacteria (e.g. Kaposi's sarcoma herpesvirus (KSHV) and *Helicobacter pylori*), and in sub-Saharan Africa, up to 33% of all cancers are attributable to infections ([Plummer et al. 2016](#)). The long-term tracing of genetic adaptations and rates of evolutionary change are therefore highly informative in understanding how a pathogen becomes virulent or transmissible, providing insights into how we can effectively manage future epidemics ([A Boire et al. 2014](#); [Andam et al. 2016](#)). In addition to a long list of known vectors and pathogens responsible for epidemic and pandemic influenza, cholera, Ebola, plague, Rift Valley fever, Yellow fever, babesiosis and tuberculosis, the influence of increasingly warmer global temperatures on the re-emergence and prevalence of novel bacterial and viral pathogens is cause for great concern ([Wu et al. 2016](#)). This realisation validates the potential of information derived from palaeopathogenic research on sub-Saharan African archaeological contexts.

### **Ancient African pathogens: Is DNA recovery possible?**

There are numerous reasons to study infectious diseases in human prehistory, but this relies on evidence of the disease persisting in the archaeological record. The likelihood of detecting ancient diseases poses significant complications as most diseases are invisible in the archaeological record. Individuals who succumb to death shortly after disease onset will not display skeletal indications of infection, while those that did survive long after the onset of symptoms might, in some instances, have developed skeletal indications of disease (Brothwell 2012). Even those that do affect human skeletal morphology (e.g. *Yersinia pestis*, *Mycobacterium tuberculosis*, *M. leprae*, *Treponema pallidum*, *Brucella melitensis*, *Plasmodium falciparum*, *Trypanosoma cruzi*) are often misdiagnosed. Taphonomic alterations also mimic disease conditions that can induce interpretation errors (pseudo-pathologies), even for experienced palaeo-pathologists (Dutour 2008). Thus, and on account of this ‘osteological paradox’ (Wood et al. 1992), disease incidence is often unnoticed or misinterpreted, and this can lead to unverified statements that some diseases were either rare or non-existent in prehistory. Unless detected with innovative archaeometric techniques such as X-ray synchrotron micro-tomography (Odes et al. 2016; Randolph-Quinney et al. 2016) or molecular (DNA) analyses, evidence of ancient disease incidence is basically imperceptible.

The application of state-of-the-art molecular analytical techniques to archaeological remains has transformed hominin evolutionary research. Examples of developments in the field of ancient DNA (aDNA) includes the recovery of aDNA from equid remains dated to ~700 ka (Orlando et al. 2013), the sequencing of the oldest hominin nuclear DNA from Sima de los Huesos (Spain) dated to 430 ka (Meyer et al. 2016) and the oldest-known *H. sapiens* genome which was extracted from a human femur recovered from the banks of the Irtysh River in Siberia, dated to 45 ka (Fu et al. 2014). These techniques have also been applied to the emerging field of apDNA and have contributed significantly to our understanding of historical epidemiological etiology (Schuenemann 2013; Devault et al. 2014; Bos et al. 2015; Harkins & Stone 2015; Rasmussen et al. 2015)

The detection of pro-viral sequences (human T-cell lymphotropic virus type I (HTLV-I)) integrated in the genomes of a 1500 year-old Andean mummy (Li et al. 1999), and sequences from the human endogenous retrovirus K (HERV-K) in Neanderthal and Denisovan genomes exemplifies the utility of molecular analytical techniques (Agoni et al. 2012). Ultimately, such ‘markers’ can be used to study the migration and co-evolution of (prehistoric) humans and their pathogens. For example, Agoni and colleagues (2012) detected that one HERV-K provirus sequence was shared by Neanderthals and Denisovans, providing confirmation that they shared a common ancestor (Reich et al. 2010). Other



relevant examples include the reconstruction of the bacterial genome responsible for the Black Death in the Middle Ages (*Y. pestis*) (Bos et al. 2011) and the near-complete genome of the medieval leprosy agent *Mycobacterium leprae* (Schuenemann 2013). These studies, however, rely on the destructive sampling of preserved human skin (Li et al. 1999), dental pulp (Bos et al. 2011) or bone (Agoni et al. 2012) for aDNA extraction and sequencing. Because ancient human remains are rare and therefore valuable, it is necessary to consider the analyses of alternative sources of human aDNA and apDNA. In this regard, ancient human (and animal) coprolites and also anthropogenic sediments (soils derived from caves and rock-shelters inhabited by prehistoric humans) present an unexplored and potentially highly viable alternative.

Extracting aDNA from ancient sediments is dependent on various post-depositional processes. Specifically, extremely cold and highly arid conditions are most suited to the preservation of aDNA. In fact, all the oldest known examples of sedimentary aDNA have been recovered from permafrost environments (Thomsen & Willerslev 2015). The discovery and re-animation of two 30,000-year-old viruses (*Pithovirus sibericum* and *Mollivirus sibericum*) from Siberian permafrost (Legendre et al. 2015) not only highlights the preservative capacity of frozen environments, but also illustrates the imaginable severity of the impact that an increasingly warmer world might have on pathogen prevalence. Similarly, the study by Bellemain and colleagues (Bellemain et al. 2013) on the palaeodiversity of fungi in arctic permafrost has detected multiple sequences related to known plant and insect pathogens.

This obviously does not sound encouraging for African aDNA studies, particularly when attempting to track down ancient apDNA and correlate these instances with human evolutionary processes. Ancient biomolecules have however been recovered from warm tropical environments. Currently, the oldest known palaeo-protein sequences are dated to 3.8 mya and originate from ostrich eggshell fragments excavated in Tanzania (Demarchi et al. 2016). In addition, the genome of a 4,500 year-old man has recently been sequenced from a cave in Ethiopia (Llorente et al. 2015). Similarly, the sequencing of tropical aDNA from ~1000 year-old extinct tortoise shells has led to the near-complete reconstruction of ancient tortoise mitochondrial genomes (Kehlmaier et al. 2017). This specific study provides proof that, under specific micro-environmental conditions (e.g., anoxic deposits and under relatively stable temperatures) can preserve aDNA for long periods in tropical environments. This is promising for African aDNA research as the caves where our ancestors lived also present conditions suitable for aDNA conservation. For example, the preservation of organic (non-fossilised) human remains comprising the oldest modern human burial (dated to >70 ka) from Border Cave (D'Errico & Backwell 2016), and the preservation of

40,000 year-old antelope (*Damaliscus niro*) horn ([Thackeray & Brink 2004](#)) at Wonderwerk Cave in South Africa, suggest that sheltered and extremely arid sedimentary conditions could contribute to the preservation of aDNA in ancient African archaeological contexts.

Many bacterial, fungal and parasitic pathogens have been isolated from archaeological contexts ([Mitchell 2013](#)); some examples are summarised in Table 1. But while the DNA of bacteria and fungi and the remains of parasitic eggs are likely to be detected in ancient African cave sediments, viral DNA is not as likely to be preserved. Unlike the double-stranded DNA structures of bacteria, viral genetic information is encoded in a variety of structures, including double- or single-stranded DNA or RNA genomes. Viral aDNA is more likely to preserve than viral aRNA because DNA degrades more slowly over time than RNA, except when integrated in their host's genome ([Li et al., 1999](#); [Agoni et al., 2012](#)) as for example HHV-6 ([Arbuckle et al. 2010](#)). Double-stranded viral DNA can furthermore be sequenced along with host aDNA in a single reaction, without additional reverse transcription or steps such as preparation of a single-stranded library ([Houldcroft et al. 2017](#)). Ancient single-stranded or RNA genome viruses in archaeological samples may occur when preservation conditions are exceptional ([Guy 2014](#)), for example in caves which have a cool and constant temperature, or where soft tissue has been preserved, such as the stomach contents of the Tyrolean Iceman ([Maixner et al. 2016](#)). While the hepatitis B virus, which causes hepatocellular carcinoma, has been recovered from human mummified remains, and may be much older than previously estimated ([Littlejohn et al. 2016](#)), the likelihood of recovering ancient viral RNA is largely predicted as there is currently little data to support this theory. Scientists have however speculated that hepatitis C virus, despite its single-stranded RNA genome, may preserve in archaeological remains as it has been detected in the tooth pulp of living humans with HCV, and HCV RNA may therefore be preserved in teeth after death ([Siravenha et al. 2016](#)).

### **Extracting aDNA and apDNA from African contexts**

In Africa, the extraction of aDNA from archaeological samples has proven challenging, largely because of an extreme shortage of appropriate aDNA extraction facilities. Internationally, there are more than 65 laboratories dedicated specifically to aDNA research (<https://palaeogenomics.wordpress.com/ancient-dna-labs/>). Yet, excluding Antarctica, Africa is the only continent on which only a single aDNA laboratory (at the Egyptian Museum in Cairo) exists. Extracting and sequencing aDNA necessitates access to highly specialised research facilities and involves strict analytical protocols and complex bioinformatic tools ([Willerslev & Cooper 2005](#); [Der Sarkissian et al. 2015](#); [Llamas et al. 2017](#)). This is largely a result of the fact that, owing to post-mortem decay, aDNA molecules are typically short (less than 100 base-pairs in

**Table 1**

<b>Pathogen</b>	<b>Modern nucleic acids</b>	<b>Ancient nucleic acids</b>	<b>Fossils</b>
RNA viruses	Primate lentiviruses HTLV (Compton et al. 2013); HCV, tooth pulp (Siravenha et al. 2016)	Plant pathogens (Barley Stripe Mosaic Virus) [Smith, Clapham, 2014] and tomato mosaic tobamovirus RNA in ancient glacial ice. Polar Biol [Castello et al, 1999]	
DNA viruses	Papillomaviruses, herpesviruses, adenoviruses, polyomaviruses [Houldcroft, 2017]	Coprolite {Appelt}, Smallpox (variola virus), permafrost (Biagini et al., 2012)	
Bacteria	Coalescent analysis of modern genetic diversity (Achtman 2016)	MTB, Y. pestis (Bos et al. 2011; Rasmussen et al. 2015); oral microbiome (Adler et al. 2013); cholera from 1849 preserved intestine {Devault, 2014}; syphilis (T. pallidum) [Montiel, 2012]	
Parasites	Body and hair lice ( <a href="#">Boutellis et al. 2014</a> ), trematode blood flukes ( <a href="#">Crellen et al. 2016</a> )	Helminth egg aDNA [Loreille 2001]; malaria aDNA from blood slides [Gelabert, 2016] and Roman-era teeth [Marciniak, 2016]	Eggs ( <a href="#">Mitchell 2013</a> )

length), physically damaged (with specific fragmentation and base modification patterns) (Willerslev & Cooper 2005) and, most problematically, that ancient samples are susceptible to contamination by modern DNA derived from environmental sources such as modern sedimentary aDNA, airborne bacterial and fungal spores, microorganisms derived from the human scientists handling the samples and also contaminated sampling equipment and laboratory spaces (Hofreiter et al. 2001; Willerslev & Cooper 2005; [Llamas et al. 2017](#)).

**Box: Why are aDNA laboratories so special?**

*Before entering any aDNA-dedicated facility, stringent sampling protocols must be followed to avoid the contamination of the ancient samples with modern DNA or nucleases (Willerslev & Cooper 2005). The former contaminant would out-compete aDNA molecules during the sequencing reaction and the latter would either digest or destroy aDNA molecules. Appropriate sampling procedures are detailed in (Llamas et al. 2017). Briefly, researchers performing aDNA sampling activities should wear full body DNA-free protective gear including face-masks, gloves, biologically-impervious body-suits and shoe-covers. All the tools used to collect each individual sample should be treated with specific chemicals that degrade exogenous DNA on their surfaces, such as the use of a 3% bleach-solution or commercial products such as DNA-Away. Importantly, no modern DNA should ever be allowed to enter the aDNA laboratory. This implies that aDNA specific laboratories must a) be physically isolated from any laboratories working with modern DNA, b) be cleaned and decontaminated after each analytical session and treated with UV-lights and c) that access must be restricted only to researchers that have been trained in aDNA analyses and workflow protocols (Llamas et al. 2017). Willerslev and Cooper (Willerslev & Cooper 2005) also provide aDNA laboratory operational protocols to follow to evaluate the validity of any aDNA-based research results.*

Given the fact that southern and also eastern Africa forms the focus of human evolutionary research, one would expect the continent to play a key role in the discovery and analyses of aDNA and apDNA. This is even more emphasised by the fact the first ever aDNA sequences studied in the early 1980s originated from the Quagga (*Equus quagga*), an extinct southern African zebra subspecies (Higuchi et al. 1984). While the current shortage of aDNA facilities in Africa leads to international collaboration, it still necessitates the acquisition of substantial funding which limits the usage and development of local expertise. While collaborations undoubtedly increase the quality of research, the lack of aDNA facility in southern Africa impedes the development of local research expertise and knowledge in a highly active and innovative scientific field that produces high impact and sometimes revolutionary research (Reich et

al. 2010; Prüfer et al. 2014; Llorente et al. 2015). Until archaeologists and scientists manage to resolve the problems concerning access to local African aDNA facilities, fragmented aDNA and poor aDNA preservation, understanding African pathogen evolution will depend on studying extant pathogen genomes and using phylogenetics to work backwards in time.

### **Pathogens and migration**

Pathogens (e.g. *Helicobacter pylori* and *Mycobacterium tuberculosis*) and also conspecific human parasites (e.g. human lice) have been used to track human population movements and have provided invaluable information regarding human migrations out of sub-Saharan Africa (Moodley et al. 2012; Comas et al. 2013) and into the New World in particular (Raoult et al. 2008). But exactly which species were brought from Africa to the rest of the world after *H. sapiens* left the continent at c. 100 ka, and again at c. 65 ka, remains unclear. While many of the major modern human diseases that originated in Africa (Wolfe et al. 2007; Houldcroft & Underdown 2016) exerted a profound influence on human evolutionary history, most are still implicated in the deaths of millions of people annually.

Importantly, different pathogens tell stories on different timescales. Large bacterial and parasitic genomes can tell very old stories as these tend to contain substantial amounts of molecular and genomic information, and many might have deep coalescent times. Smaller viral genomes, especially viruses which mutate rapidly (e.g. RNA viruses), 'turn over' their viral genomes so fast that ancient variation has all been lost or replaced before we can observe it (Biek et al. 2015). Such instances however present an opportunity to study more recent pathogen population histories on shorter present-day time scales.

### **Three stories of migration and disease from Africa**

#### **1. HIV, colonialism and male migration**

The zoonotic origins of HIV have been reviewed thoroughly elsewhere (Hemelaar 2012). What is of interest is the story of changing human behaviour and migration patterns that allowed HIV to spread and become a global pandemic. Pandemic HIV is caused by viruses from group M, which jumped from wild chimpanzees in Cameroon to humans some 150 years ago, although primate (especially the great apes, i.e. gorillas, chimpanzees and bonobos) to human transmission of SIVs must have been occurring for hundreds and possibly thousands of years before this. This likely resulted in infections which were poorly adapted to their new human host and therefore unable to spread efficiently between humans. In

Cameroon the virus did eventually adapt to spread from human-to-human, and then began to be transmitted southwards across Africa, beginning its journey along the route of the Sangha river to Kinshasa in the Democratic Republic of the Congo (DRC). The transmission of HIV along this route was likely driven by German colonialism in Cameroon which promoted increased movement of goods such as rubber (and also HIV-positive individuals) into the DRC by river. This period of colonial rule is a plausible explanation for the dating and location of the most recent common ancestor (MRCA) of HIV group M strains derived from Kinshasa in around 1920 (Faria et al. 2014).

Kinshasa became the epicentre for the HIV pandemic and, within 20 years, HIV had spread to Brazzaville, Lubumbashi and Mbuji-Mayi. Increasing population mobility due to urbanisation and new transportation methods, such as railways, further facilitated the spread of HIV. In fact, historical data on population movements by rail and river supports data derived from modelling the spread of HIV genetically. HIV reached cities receiving high volumes of rail and river migration from Kinshasa earlier (by an estimated 15-20 years) than cities which received lower volumes of river traffic from Kinshasa. A disease which had once been transmitted only sporadically, first from primates to bush-meat hunters in tropical Africa, has now become a fully human-adapted disease free to infect residents in densely populated areas with increasing mobility, allowing it to spread across Africa (Faria et al. 2014).

International mobility was instrumental in the development of HIV from an epidemic to a pandemic. Human migration was at the heart of the early global spread of HIV, and this is reflected in the genetic structure of HIV strains collected from current and historic HIV cases. Professionals from Haiti who travelled back and forth to post-colonial Congo carried a specific lineage of HIV group M (subtype B) with them; subtype B HIV was detectable in Haiti by 1964. From Haiti, subtype B was able to spread to the USA (Faria et al. 2014) around 1970, 10 years before the first US cases were recognised (Worobey et al. 2016). While many aspects of human behaviour are also integral to the spread of HIV (such as bush-meat hunting, sex work, and unsafe medical practices that led to extensive needle reuse), migration over smaller and larger geographic distances, facilitated by mass transportation and trade, enabled HIV group M to spread widely and rapidly.

## 2. New ecological niches: flukes, fishing and farming in the African Pleistocene

*S. mansoni* is a trematode blood fluke found across sub-Saharan Africa, the Caribbean and parts of South America, infecting 250 million people worldwide and killing a small proportion of infected individuals

every year by causing chronic inflammation of the spleen and/or liver. *S mansoni* requires an intermediate freshwater snail host to complete its life cycle, which means that populations who engage in behaviour which leads them to spend periods of time wading in fresh water are at risk of infection. There are a number of different behaviours which bring humans in to contact with *S mansoni*, including wading through irrigated fields ([Hibbs et al. 2011](#)), which lead to human *S mansoni* infections in ancient Nubia, and also fishing at the edge of fresh water lakes and rivers ([Crellen et al. 2016](#)), a food exploitation technique which dates to between 74-111KYA ([Yellen et al. 1995](#); [Brooks et al. 1995](#)) for freshwater resources and even earlier for marine resources (152–176KYA) ([Marean et al. 2007](#)).

The origins of *S mansoni* as a human infection are intimately tied up with human migration and changing human behaviour. *S mansoni*'s closest relative is *S rodhaini*, a rodent trematode, and it is likely that the last common ancestor of these two species was a rodent parasite. This ancestor was able to switch hosts and infect humans following changing human behaviour, leading to the speciation of the trematode ancestor into *mansoni* and *rodhaini* around 125kya. The most genetically diverse isolates of *S mansoni* come from lakes Victoria and Albert in Uganda, suggesting that it was in east Africa – where some of the earliest anatomically modern human remains are found ([McDougall et al. 2005](#)) – that *S mansoni* was able to switch hosts, likely after humans began to exploit fresh water lakes and rivers through fishing and dwelling on the edges of lakes to hunt the fauna who came there to drink

The spread of farming in Africa led to movements of people and the spread of technology, and this in turn helped to spread *S mansoni* across sub-Saharan Africa. Isolates of *S mansoni* from east and west Africa were a single population (likely endemic in east Africa, where the original host-switch in AMH occurred) until 7KYA. This could reflect a movement of infected people as part of the expansion of farming and pastoralism ([Marshall & Hildebrand 2002](#)), and the population expansions around 6KYA of the Yoruba and Luhya ([Crellen et al. 2016](#)).

Genetic data also charts the path of *S mansoni* from Africa to its other foci. The genetic diversity of *S mansoni* in the Caribbean reflects spread of this parasite during the trans-Atlantic slave trade. *S mansoni* found in Guadeloupe diverged from *S mansoni* from Senegal and Cameroon between ~1100-1750CE. This coincides with the beginning of the French colonial slave trade to the French Caribbean, from 1669-1864. The mass enslavement, forced migration and then forced labour in the French Caribbean of at least 20,000 West Africans therefore seems the most plausible explanation for the spread of *S mansoni* to the Caribbean ([Crellen et al. 2016](#)).

### 3. Chimpanzees, hominins and herpes

Herpes simplex virus 2 (HSV2) is a sexually transmitted human DNA virus that causes genital lesions and, rarely, encephalitis ([Tang et al. 2003](#)) and is associated with increased risk of HIV acquisition (Freeman et al. 2006). After primary infection, the virus adopts a life cycle of latency punctuated by periods of lytic replication when new hosts can be infected through genital contact. The virus is related to the human oral pathogen herpes simplex 1 (HSV1). Both are alphaherpesviruses, which are found in many primates ([Wertheim et al. 2014](#)).

HSV2 was originally thought to have co-speciated with humans when our lineage diverged from chimpanzees and bonobos, but recent comparisons of the HSV1, HSV2 and chHV1 genomes suggests that HSV2 is in fact more closely related to chimpanzee herpesvirus than human herpesvirus (Wertheim et al. 2014). A similar pattern can be seen between genetically distinct clades of *Pediculus humanus* (the human head and body louse), with clade divergence times which pre-date the emergence of the *Homo sapiens* in Africa and unusual geographic distributions ([Reed et al. 2004](#)). Taken together, these data are highly suggestive of close bodily interaction between hominin species (Ashfaq et al. 2015).

The analysis of human and chimp simplex viruses found that HSV2 diverged from ChHV1 between 1.4 and 3 MYA, and an intermediate hominin is inferred to have served as a host for proto-HSV2 before it introgressed into the ancestors of modern humans. Humans are susceptible to infection by primate simplex viruses from bite injuries, suggesting that hunting of chimpanzees by hominins could have been one transmission route. However, it is unclear how recently HSV2 introgressed into the modern human population (and whether that transmission was sexual or the result of bite injuries during hunting), but given HSV2's global distribution, it seems likely that this virus infected the human lineage before the migration of AMH out of Africa. There is evidence of interbreeding and genetic introgression between anatomically modern humans and unknown hominins in Africa around 35kya ([Hammer et al. 2011](#); Hsieh et al. 2016), too late to be the HSV2-transmitting hominin; but together, these different lines of evidence illustrate that interbreeding between different groups of hominins, not all known from fossils, was occurring across the globe in the Pleistocene. It also predicts certain patterns of migration, as different hominins interacted as climate conditions changed, changing resource distribution and generating the potential for inter-species conflict.



Unfortunately, ancient DNA evidence will not resolve the identity of the hominin(s) who transmitted proto-HSV2 to the ancestors of modern humans. There is an 'event horizon' for ancient DNA preservation, predicted by factors such as the age of the fossil, the heat and humidity it is exposed to, and the potential for degradation by microbes (Allentoft et al. 2012). This means that recovering authentic aDNA or apDNA from an African fossil more than 1 million years old is highly unlikely.

The issue is further complicated by the high human-to-virus DNA ratios experienced when trying to sequence herpesviruses from living humans, meaning only a tiny proportion of the total DNA within a sample would come from HSV2 (Houldcroft et al. 2017) if samples from archaeological specimens were available; explicit enrichment of HSV2 DNA by PCR or target capture would be required (Houldcroft & Breuer 2015; Depledge et al. 2011; Ebert et al. 2013).

However, this is not the end of the story: the examples of HIV and *S mansoni* demonstrate the power of genomic analysis to reveal aspects of human and pathogen co-evolution. Computational modelling is increasingly being applied within archaeology to reconstruct past events (eg (Crema et al. 2016; Bortolini et al. 2016)), uniting many data sources. For example, modelling has been used to predict the movement of anatomically modern humans out of Africa based on climate data and patterns of extant human genetic diversity, without relying on fossil or other archaeological data (Eriksson et al. 2012). Computational modelling and knowledge of areas of Africa with particular importance for the understanding of human evolution would allow for more HSV2 genomes to be collected from humans and ChHV in an evolutionarily informed manner and the potential transmission route reconstructed (Underdown et al. 2017). This would allow researchers to focus on areas of Africa where particularly ancient HSV2 lineages are predicted to be found; sequencing of a bonobo herpes simplex genome would also aid in reconstructions of the history of ChHV1 and HSV2.

## Conclusions

It is evident that ancient biomolecular research can contribute to existing genome databases which may have public health benefits by providing tools for developing therapeutics, particularly if virulent forms of ancient diseases re-emerge. This is important as history has taught us that disease is by far the most effective eradicator of our species. Past pandemics are much more than just ancient history. They are important drivers of human genetic diversity and natural selection (Pittman et al. 2016). At the time of writing this Review, a report entitled 'Killer bird flu has spread across Europe - are humans next?' appeared in New Scientist (<https://www.newscientist.com/article/2113725-killer-bird-flu-has-spread->

[across-europe-are-humans-next/](#)). While rather sensationalist, the H5N8 virus, lurking in domestic and wild avian populations since 2014, has rapidly spread along avian migration routes into India, the Middle East and Europe and it certainly does hold the potential to develop into a global influenza pandemic. The potentially severe economic and social repercussions of disease epidemics are further demonstrated by both historical (e.g. plaque, smallpox, influenza etc.) and current (i.e. Zika, Ebola, SARS etc.) examples. But the biological origin of a many prehistoric, historical and even contemporary pathogens remains mysterious. The emphasis should therefore also be on the development of sub-Saharan capabilities to detect, predict, prevent and control potential infectious disease epidemics rather than waiting for known diseases to threaten global human health. This is particularly important given the current global interconnectedness, which can put people at risk of diseases that emerge in distant locales.

The recent retrieval of the first ancient African genome from Mota Cave in Ethiopia (Llorente et al. 2015) dated to c. 4,500 years suggests that the prospect of retrieving both human and aDNA from sub-Saharan African contexts is becoming progressively more promising. Temperate and Arctic regions have yielded more aDNA sequences than tropical regions, partially because conditions are more favourable to the preservation of aDNA, but also because they have been researched more intensively (Slatkin & Racimo 2016). Because of the paucity of aDNA sequences from Africa, any novel pathogen genomes will provide novel revelations concerning human-pathogen co-evolutionary processes. As this review has shown, evidence from even modern African pathogen genomes can shine a light on changes in human behaviour and migration. The unique combination of an unrivalled archaeological record and a thriving and highly skilled academic community places southern African archaeologists, geneticists and medical scientists in a prime position to explore past pathogenic influences and to contribute to the improvement of human quality of life and longevity.

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