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Title: Neanderthal Genomics Suggests a Pleistocene Time Frame for the First Epidemiologic Transition

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Charlotte J. Houldcroft[1,2] and Simon J. Underdown[3]*

*Corresponding author: sunderdown@brookes.ac.uk

Author affiliations:

1. UCL Institute of Child Health, Guilford St, London, WC1N 1EH, UK
2. Division of Biological Anthropology, Department of Archaeology & Anthropology, University of Cambridge, Pembroke St, Cambridge, CB2 3QG, UK.
3. Human Origins and Palaeoenvironmental Research Group (HOPE), Department of Anthropology & Geography, Oxford Brookes University, Oxford OX3 0BP, UK

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Abstract – 246 words

High quality Altai Neanderthal and Denisovan genomes are revealing which regions of archaic hominin DNA have persisted in the modern human genome. A number of these regions are associated with response to infection and immunity, with a suggestion that derived Neanderthal alleles found in modern Europeans and East Asians may be associated with autoimmunity. As such Neanderthal genomes are an independent line of evidence of which infectious diseases Neanderthals were genetically adapted to. Sympathetically, human genome adaptive introgression is an independent line of evidence of which infectious diseases were important for AMH coming in to Eurasia and interacting with Neanderthals. The Neanderthals and Denisovans present interesting cases of hominin hunter-gatherers adapted to a Eurasian rather than African infectious disease package. Independent sources of DNA-based evidence allow a re-evaluation of the first epidemiologic transition and how infectious disease affected Pleistocene hominins. By combining skeletal, archaeological and genetic evidence from modern humans and extinct Eurasian hominins we question whether the first epidemiologic transition in Eurasia featured a new package of infectious diseases, or a change in the impact of existing pathogens. Coupled with pathogen genomics, this approach supports the view that many infectious diseases are pre-Neolithic, and the list continues to expand. The transfer of pathogens between hominin populations, including the expansion of pathogens from Africa, may also have played a role in the extinction of the Neanderthals and offers an important mechanism to understand hominin-hominin interactions well back beyond the current limits for aDNA extraction from fossils alone.

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Current models of infectious disease in the Pleistocene tell us little about the pathogens that would have infected Neanderthals. If we consider the work of Cockburn (Cockburn 1963; Cockburn 1971), Omran (Omran 1971), and Barrett (Barrett et al. 1998) who argue that infectious disease only started to seriously impact human groups after the development of agriculture during the Holocene, making inferences about the pathogens which affected the Neanderthals and other Pleistocene Eurasian hominins is difficult. In the first epidemiologic transition model (FET) as originally formulated, Pleistocene hunter-gatherers such as the Neanderthals should not be at risk from the majority of "pestilences", as these pathogens were acquired from domesticated and peri-domesticated animals (Armelagos and Harper 2005). The FET model as developed by researchers such as Armelagos and Harper (Armelagos and Harper 2005) stresses a significant increase in the mortality caused by infectious diseases with changing living conditions connected with the rise of agriculture, increased sedentism and higher population densities. Much focus is therefore placed on an era tens of thousands of years after the Neanderthals became biologically extinct. However, new genetic evidence from hominin and pathogen genomes has the potential to change our view of Neanderthal infectious disease pathology. In turn, this evidence helps us to understand the infectious disease landscape that *Homo sapiens*, a hominin adapted to a landscape of African pathogens, might have encountered in Eurasia – tens of thousands of years before the beginnings of agriculture. This will further enrich recent advances to the FET model.

Firstly, we must consider the current tools for studying infectious disease in the Pleistocene. Before the advent of ancient DNA sequencing methods, researchers were limited to studying the skeletal pathologies (fossilised evidence of bones responding to infection and inflammation) of humans and Neanderthals from this epoch. However, only a limited subset of infectious diseases leave behind these lesions. The publication of high-quality Neanderthal and Denisovan genomes gives us a new opportunity to study Pleistocene infectious disease. As a result of making comparisons between modern human genomes, seeking genetic polymorphisms which vary in function or frequency between populations, and by also comparing human genomes with high-quality Denisovan and Neanderthal genomes, we are beginning to find evidence of introgressed Neanderthal and Denisovan

77 alleles and haplotypes which have functions in immunity and the response to infection
78 (Prüfer et al. 2014; Sankararaman et al. 2014; Vernot and Akey 2014). Some of these
79 polymorphisms show evidence of positive selection, often in individual populations (Racimo
80 et al. 2015) fuelling the hypothesis that these stretches of introgressed Neanderthal or
81 Denisovan DNA have persisted because they increased the fitness of anatomically modern
82 humans (AMH) when dispersing into new environments (adaptive introgression, reviewed in
83 (Segurel and Quintana-Murci 2014)). Researchers from a range of disciplines interested in
84 the evolution of the modern suite of infectious disease can also draw inferences from this
85 new source of data: previous studies have compared the genomes of humans and extant
86 great apes to understand the evolution of primate lentiviruses (eg simian immune deficiency
87 virus, the ancestor of HIV) (Compton et al. 2013; Lim et al. 2010; Sauter et al. 2011) and
88 herpesviruses (Aswad and Katzourakis 2014). The genomes of many pathogens themselves
89 can be used to infer their evolutionary history and that of their hosts (for example, lice
90 (Boutellis et al. 2014; Weiss 2007; Weiss 2009), malaria parasites (Holmes 2010; Liu et al.
91 2010) and herpesviruses (McGeoch et al. 2006)). Furthermore, ancient DNA technology now
92 encompasses pathogen DNA, and in the future it will be possible to sequence some
93 pathogen DNA directly from Neanderthal remains – including pathogens that do not cause
94 skeletal lesions, for example the Neanderthal oral microbiome. Through comparisons of
95 host and pathogen genetic data with skeletal evidence of infection, it is increasingly possible
96 to analyse which pathogens shaped the evolution of modern humans and their closest
97 relatives, and the antiquity of these infections in hominins.

98 We will discuss the evidence for infectious disease in Neanderthals, beginning with that of
99 infection-related skeletal pathologies in the archaeological record, and then consider the
100 role of infection in hominin evolution. We have a synthesised current thinking on the
101 chronology of emergence of notable European disease packages (Table 1). Finally, we will
102 consider how this evidence may be integrated into the FET model.

103 We believe that new genomic evidence from modern humans, pathogens, and extinct
104 hominins can be brought together as a set of minimal, testable hypotheses about the FET.
105 They are as follows:

106 • An increasing number of diseases characterised as part of the Holocene Neolithic

disease package will be shown to have been human pathogens in the Pleistocene

- aDNA and comparative genomics will provide evidence of pathogen transfer between AMH and other Eurasian hominin groups
- aDNA and comparative genomics will identify further examples of introgressed Neanderthal DNA buffering the impact of a Eurasian disease package on AMH colonising Eurasia

If these hypotheses are confirmed, we would reformulate the FET to include a longer 'burn in' period, pre-dating the Holocene and the introduction of agriculture, in which AMH migrating in to Eurasia faced the selective pressure of a new temperate infectious disease package, including pathogens which had to some extent co-evolved with local hominins such as Neanderthals and Denisovans. We speculate that the introgression of immune-related loci into modern human genomes demonstrates the adaptation of Eurasian hominins to Eurasian diseases, and the selective advantage gained by admixed AMH. We explore the evidence for these hypotheses below.

The Neanderthal Fossil Record

Neanderthals were large bodied hominins that inhabited Eurasia widely from approximately 250,000 to 28,000 years ago (Davies and Underdown 2006). Neanderthals occupied a hunter-gatherer subsistence niche, forming small bands of approximately 15-30 individuals (Davies and Underdown 2006). Archaeological analysis suggests that while Neanderthal groups were relatively self-sufficient there was some level of exchange and transfer of materials (Hayden 2012). The Neanderthal fossil record of some 400 individuals represents one of the largest collections of extinct hominin remains and is larger than that of contemporary Pleistocene *Homo sapiens* fossils. Numerous studies have attempted to estimate or model Neanderthal population size based on methods ranging from analyses of archaeological materials to aDNA, mtDNA and mathematical modelling with mixed degrees of effectiveness (Bocquet-Appel and Demars 2000; Fabre et al. 2009; Ghirotto et al. 2011; Green et al. 2006; Green et al. 2008). Neanderthal aDNA data suggests smaller effective population sizes, with a female Ne of 3500 (based on mitochondrial DNA sequences (Briggs et al. 2009)) and similar estimates of a small effective population size over a long period are derived from the Altai Neanderthal genome (Prüfer et al. 2014) while Harris and Nielsen

(2015) suggest the long-term effective size of Neanderthals was closer to 1000 (Harris & Nielsen, 2015). The total size of the Neanderthal fossil record is, therefore - while only a fraction of the whole – when compared with modern medical trials extremely large and it could be reasonably argued that relatively strong conclusions can be drawn from its analysis.

Neanderthal fossils are still often described and interpreted in relative isolation from one another. The effect of this approach is to highlight well known pathological specimens (Shanidar, La Ferrassie etc.) while weakening the focus on the broad pathological trends seen in the Neanderthal species as a whole (Davies and Underdown, 2008). That the Neanderthals fulfilled the criteria expected of the Pleistocene hunter-gatherers is thus taken as orthodoxy even when data for such is frequently absent. When reviewed as a population there is evidence that along with traumatic injury the Neanderthals displayed a broad range of dental pathology and degenerative diseases as well as a large amount of non-specific infection (Antón 1997; Duday and Arensburg 1991; Fennell and Trinkaus 1997; Ogilvie et al. 1998).

From the perspective of the FET as first formulated (Cockburn 1963; Cockburn 1971; Omran 1971), the Neanderthals' small group size and limited exchange networks suggests that they could not act as reservoirs for the majority of infectious diseases. As our knowledge of pathogen evolutionary history increases, combined with Neanderthal fossil evidence, we can see that a reformulated FET of diverse infectious diseases affecting Pleistocene hunter-gatherers applies equally to the Neanderthals and other Eurasian hominins. Indeed, the group-structure of Neanderthals would have made disease a potent factor in any demographic collapse related to extinction events (Underdown 2008).

Innate, adaptive and archaic immunity in hominin genomes

2010 saw the publication of the draft Neanderthal genome sequence (Green et al. 2010), which revealed that humans living outside Africa have a small proportion of Neanderthal ancestry – ~2% of their genome (Seguin-Orlando et al. 2014), and some East African individuals carry a smaller proportion of Neanderthal ancestry acquired from back migration of Western Eurasians into Africa over the last 7000 years (Busby et al. 2016; Llorente et al.

2015). Genome sequences from a growing number of Neanderthals are available: a draft sequence from Vindija in Croatia, the composite sequence of DNA from bones of different individuals from three different layers, dating from between 38-45kya (Green et al. 2010); a low-coverage sequence of a Neanderthal found in Mezmaiskaya in the Caucasus, from a layer dated as 60-70kya; and a high-quality Neanderthal genome from the Altai region (Prüfer et al. 2014), dated to 29-45kya. The data set is growing constantly, bolstered by a 49kya Neanderthal exome sequence (the protein-coding ~1% of the genome) from El Sidron in Spain, and a further 44kya exome from Neanderthal remains recovered from Vindija (Castellano et al. 2014). Comparisons of these genome and exome sequences to those of modern humans have identified several regions of genetic similarity between humans and Neanderthals that are thought to have arisen from admixture between these two hominins (Racimo et al. 2015). As the number of archaic hominin genomes grows, researchers are able to look more systematically at these regions of similarity. Approaches to identifying introgressed Neanderthal regions in the human genome which may be adaptive have looked for a range of different kinds of variation, from haplotype blocks hundreds of kilobases long, to single nucleotide polymorphisms (SNPs).

There is evidence for Neanderthals contributing to the immune system in some modern humans, and here we discuss some recent examples which fall in to one of two categories: introgressed alleles where there is evidence of interaction between the locus and a pathogen (or pathogen vector) which also has evidence of being present in Pleistocene Eurasia; and examples of introgressed Neanderthal alleles which have evidence of having been positively selected for since admixture occurred. We believe these examples are most informative about the genetic selection pressures of the disease landscape Eurasian AMH were exposed to.

The adaptive introgression of Neanderthal HLA alleles into modern humans has been addressed in detail elsewhere (Abi-Rached et al. 2011; Parham and Moffett 2013), but the HLA is not the only region of the human genome important for infection and immunity. A haplotype containing *OAS1*, *OAS2*, *OAS3* of Neanderthal origin has been found in some modern human genomes (Mendez et al. 2013): these genes activate RNase L to degrade viral RNA. The introgressed allele of SNP rs15895 prevents a truncated form of the *OAS2* protein (present in some modern humans) and may alleviate symptoms to tick-borne

encephalitis virus (TBEV) disease in Europeans (Barkhash et al. 2010). TBEV is found in forested areas of northern, central and eastern Europe, which would have formed a major part of the Neanderthals' typical ecosystem (Davies and Underdown 2006; Stewart 2006). Phylogenetic analysis has dated the divergence of the mammalian TBEV family to between 16 and 45kya, based on extant Eurasian lineages, suggesting some temporal overlap between multiple Eurasian hominin populations and these pathogens (Heinze et al. 2012). However, Mendez et al (Mendez et al. 2013) do not find explicit support for adaptive introgression the OAS locus, only support for introgression.

Sankararaman and colleagues (Sankararaman et al. 2014) scanned the genomes of Europeans and Asians for evidence of individual SNPs that have introgressed from Neanderthals, a number of which have been associated with immunity and auto-immunity in modern humans. One of the most interesting results was in interleukin 18 (*IL18*), a gene with a central role in the innate immune response and the development of bacterial sepsis. IL18 induces interferon gamma, which can protect against infection; but increased IL18 cytokine signalling is also associated with allergic reaction and development of sepsis (Dinarello and Fantuzzi 2003). The introgressed *IL18* SNP rs1834481 is associated with decreased serum IL18 levels. If Neanderthals were particularly at risk from bacterial sepsis, this could have created a selection pressure for reduced *IL18* expression (He et al. 2010).

High levels of population differentiation in Toll-like receptor cluster *TLR6-TLR1-TLR10* indicated to Danneman and colleagues (Dannemann et al. 2016) that there was evidence of repeated archaic introgression at this locus, with similarity of two haplotypes to Neanderthal haplotypes, and a further haplotype found in modern humans showing greatest similarity to a Denisovan haplotype. These haplotypes are present at greater frequencies than would be expected by drift alone. Genes of the innate immune system were also a focus for Deschamps and colleagues (Deschamps et al. 2016), and their work on positively-selected genomic regions highlighted the same *TLR6-TLR1-TLR10* gene cluster as a likely introgressed Neanderthal haplotype in Europeans.

Toll-like receptors play an important role in the innate immune response, present on the cell surface and helping the innate immune system to detect fungi, bacteria and parasites (Akira et al. 2006). Analysis of the expression of the archaic haplotypes in lymphoblastoid cell lines

(Epstein-Barr virus immortalised B cells) showed that the introgressed haplotypes was associated with higher expression of *TLR6*, *TLR1* and *TLR10*. Combining SNP data from the archaic haplotypes with genome-wide association study results indicated that archaic haplotypes are associated with phenotypes such as reduced *Helicobacter pylori* seroprevalence, but also an increased risk of allergic diseases (Dannemann et al. 2016; Deschamps et al. 2016). This echoes the results of other studies of putative introgressed Neanderthal alleles, which highlighted introgressed alleles which are associated with diseases of allergy or auto-immunity in modern humans (Sankararaman et al. 2014). Interestingly, the analysis of the TLR gene cluster undertaken by Deschamps and colleagues found the introgressed Neanderthal haplotype they had concentrated on had been subject to positive selection in modern Europeans thousands of years after the hypothesised admixture event, between approximately 6000 and 13,000 years ago (Deschamps et al. 2016). This is compatible with the hypothesis that the FET was a process which included an increase in the selection pressure applied by pathogens already present in Eurasia, but which were more likely to affect fertility and mortality after the introduction of agriculture to Eurasia. Equally, new pathogens which interacted with the *TLR6-TLR1-TLR10* gene cluster may have been the source of this selective pressure.

There are regions of the genome in which Neanderthal DNA does not persist (Sankararaman et al. 2014; Vernot and Akey 2014), seemingly removed by purifying selection for disadvantageous phenotypes; the continued presence of genetic variants associated with immunity in some European and Asian genomes suggests that some Neanderthal haplotypes conferred a selective advantage to *Homo sapiens* during the colonisation of Europe and East Asia and should be described as adaptively introgressed (Racimo et al. 2015; Segurel and Quintana-Murci 2014).

Individual studies of Neanderthal-human admixture use different methods to identify introgressed DNA, and subsequently identify different regions of the human genome as Neanderthal-derived. With the growing availability of whole Neanderthal and Denisovan genomes, we can test whether immune-related variants are more represented among these variants than would be expected by chance, as has shown for genes with a role in lipid catabolism in Europeans, and immune loci in Asians (Khrameeva et al. 2014). It is also important to note that our interpretation of the function of adaptively introgressed variants,

and our identification of immunity-related variants, relies upon our knowledge of the function of genes and polymorphisms within the human genome, which is incomplete – for example, there may be many more polymorphisms affecting susceptibility to viral, bacterial or fungal infection which we have not yet identified in modern humans, and therefore cannot identify in Neanderthal genetic data. Other questions remain about how these variants may have functioned in a Neanderthal genetic background, although there is a growing scientific interest in characterising how putatively introgressed alleles alter phenotypes such as gene expression in experimental systems (eg lymphoblastoid cell lines (Dannemann et al. 2016)).

Pathogen genomics, ancient and modern

Genomes of many pathogens can be used to trace their evolutionary history, providing insights into human evolution. For some pathogens, the dates generated by this analysis seem too recent to fit with their known geographical distribution or species reservoirs (Biek et al. 2015) – direct sequencing of ancient pathogen genomes, and the footprints they have left in host genomes, can therefore be a useful way to study their early history (Aswad and Katzourakis 2012; Katzourakis 2013). The work of Johannes Krause (Bos et al. 2011b) and others (Biagini et al. 2012; Wagner et al. 2014) has demonstrated the possibility of directly testing ancient remains for evidence of infection by amplifying the DNA or RNA of the pathogens which infected them in life. As the horizon for amplifying ancient host DNA moves further back in time (eg the 400,000 year old mtDNA sequence from Sima de los Huesos in Spain (Meyer et al. 2014)), sequencing of ancient pathogen DNA from selected remains, particularly dental calculus, of Neanderthals and Denisovans is underway (Dobney et al. 2015). We are already aware of the oral pathogens afflicting Mesolithic and early Neolithic individuals (Adler et al. 2013).

Infectious disease in the Pleistocene

Our views of the infectious disease environment of the Pleistocene are heavily influenced by skeletal data and studies of contemporary hunter-gatherers (Cockburn 1971); but the paradigm of the first epidemiologic transmission must continually evolve to incorporate new

genomic data from many sources. The Neanderthals (and to a lesser extent the Denisovans) provide new ways to understand the evolutionary pressures facing the genus *Homo* during the late Pleistocene. The indigenous Eurasian Neanderthal populations had been adapting to their environment, including its infectious diseases, at least since the arrival of the ancestor of the Neanderthals (Stringer 2012) *Homo heidelbergensis* in Europe some time between 850-500,000 years ago and in the case of the Denisovans any time up to 1 million years ago. Whereas *Homo sapiens* would have been under pressure to adapt to the infectious diseases of an African environment. As infectious disease can exert strong selection pressure on hominin genomes as they enter new environments (Barreiro and Quintana-Murci 2010; Fumagalli et al. 2009; Prugnolle et al. 2005), adaptive introgression would have been an important source of genetic diversity for AMH, alongside processes such as long-term balancing selection (Segurel and Quintana-Murci 2014).

Neanderthal genomes fill an important gap in the genetic paleopathological record that has already been informed by studies of extant primates. Comparing modern human and great ape genomes helps us to understand the ancient pressures that infectious diseases have exerted on African primates. Variation in ancient and modern human genomes has revealed numerous loci associated with the immune system that seem to play a role in human evolution before and after AMH left Africa. One of the best known examples is *CASP12* (caspase-12), found in the genome of a 7,000-year-old hunter-gatherer from La Brana (Olalde et al. 2014) and in all individuals from a mixed sample of 24 pre-, early and late Neolithic humans from Spain (Hervella et al. 2012). These humans all carried the non-functional form of *CASP12*, protective against bacterial sepsis, and present at or approaching fixation in non-African populations (Xue et al. 2006). This mutation predates the origin of animal domestication in Europe (Hervella et al. 2012) based on ancient DNA data from AMH. In contrast, all of the available Neanderthal or Denisovan genomes sequenced to date carry the ancestral active form of *CASP12*. Were humans leaving Africa subject to different sepsis-related selection pressures to other Eurasian hominins, or did other hominins have different genetic adaptations to the same pressures?

*****TABLE 1 HERE*****

When genetic variation such as the loss of *CASP12* in AMH is considered alongside the

reduced expression of Neanderthal *IL18* SNP found in some Europeans and Asians, combined with earlier paleopathological evidence for oral disease (Zanolli and Mazurier 2013) and septicaemia (Gracia-Tellez et al. 2013) in Pleistocene hominin *Homo heidelbergensis*, there is a suggestion that selection pressure exerted by bacterial sepsis shaped the genomes of archaic and AMH, long before the assumed arrival of traditional zoonoses with the rise of agriculture in the Holocene. Likewise the introgression of genes with antiviral activity into modern human environments points towards viral infections afflicting European hominins to a degree strong enough to favour adaptive introgression (Segurel and Quintana-Murci 2014), protecting admixed AMH against the same pathogens which afflicted the Neanderthals. A better understanding of the function of introgressed variants will enrich our understanding of Pleistocene infectious disease.

Paleogenomics provide us with evidence that a number of pathogens (discussed below), intimately associated with the FET were likely to have been present in Eurasian hominins before the introduction of agriculture and pastoralism, and it was the relative impact of different circulating pathogens that changed in the Holocene, as much as a new infectious disease package introduced by animal domestication. Changes in the impact of pathogens after the transition to agriculture may have included increased pathogenesis. A modern example comes from studies of rabies virus strains circulating in dogs which have reduced incubation times (Yu et al. 2014) compared to strains circulating in wild animals. The finding that medieval bubonic plague isolates of *Yersinia pestis* carry no genetic changes compared to modern isolates which could explain a change in virulence also suggests increased (or decreased) morbidity and mortality in a population can occur without a change in pathogen phenotype (Bos et al. 2011a).

Studying the phylogenetic relationships of extant pathogens has led researchers to conclude that many infectious diseases have been co-evolving with humans and our ancestors for tens of thousands to millions of years. Pathogens that were traditionally thought to be zoonoses acquired from herd animals may in fact be anthroponoses, pathogens humans passed to their animals during the rise of agriculture (Kidgell et al. 2002; Wirth et al. 2008). In Table 1, we consider which infectious diseases European Neanderthal populations may have experienced. Pleistocene diseases include pathogens which are found in all primates, and are therefore likely to have co-speciated with Neanderthals (also known as heirloom

pathogens); and also those pathogens that phylogenetic evidence suggest predate the Holocene, and are therefore potential Neanderthal pathogens. The same infectious diseases would have affected the first AMH in Europe. They are compared to the diseases associated with the transition to agriculture in the Holocene. The list of pathogens with phylogenetic or paleogenomic evidence for being present in the Pleistocene is constantly growing and challenging our perceptions.

Certain pathogens are of particular interest to those studying infectious disease in Neanderthals (Table 1). Kuhn and colleagues (Kuhn et al. 2009) speculate that a Pleistocene European rock shelter shows evidence of bedding being burned to eliminate parasites. If Pleistocene European AMH were subject to parasites contaminating their bedding, Neanderthals may have been similarly burdened, as there are many helminths which parasitise African primates and some modern humans (Mitchell 2013; Ravasi et al. 2012). Neanderthals and AMH were likely to have carried these parasites, although the extent to which they would have caused symptomatic disease is less clear (London and Hruschka 2014). Phylogenetic analysis suggests that the different species of *Brucella* bacteria diverged tens of thousands of years before the origin of pastoralism, and have likely been endemic in wild animal populations for 80,000 – 300,000 years (Foster et al. 2009). There are skeletal reports of brucellosis in *Australopithecus africanus,* an order of magnitude earlier than the above estimates (D'Anastasio et al. 2011).

Neanderthals were therefore subject to a wide variety of infectious diseases, many of which do not leave skeletal lesions, although paleogenomics may allow us to study them in the future. Some pathogens can be inferred to have been Neanderthal-infecting pathogens with confidence; others have conflicting evidence in support of their pre-Holocene emergence (particularly tuberculosis, with divergent molecular, fossil and lipid biomarker (Lee et al. 2015) dating evidence). These pathogens would have had the capacity to cause morbidity and mortality in a variety of settings: infections of dental carries and flesh wounds; childhood diseases (e.g. varicella zoster - chicken pox); gastrointestinal infections; sexually transmitted infections; progressive infections such as leprosy; and many chronic infections which would have been carried for life and only become symptomatic when other infections led to immune suppression, such as tuberculosis and hepatitis.

Disease exchange

There is as yet no evidence of infectious disease transmission between AMH and Neanderthals, but when considered in the light of the temporal and geographical overlap between the two populations (Higham et al. 2014) and the evidence of admixture, it must have occurred. There is compelling evidence from Africa of pathogen exchange between humans and other hominins, preserved in the genome of human herpesvirus 8 (KSHV). The K15 gene of KSHV has three highly divergent forms, P, M and N. P is most common, M is found at low frequencies worldwide, and N is rare and found solely in Africa (Hayward and Zong 2007). It is thought that the highly divergent M and N forms of K15 introgressed into human KSHV strains through recombination with another herpesvirus that has yet to be detected in modern humans. Based on the divergence dates of the different forms of K15, Hayward and Zong suggest that the M form diverged from the P form 200,000 years ago, and the N form 500,000 years ago. The presence of these other K15 gene forms has arisen through contact with other hominins who carried their own KSHV-like viruses which speciated with each hominin group. It was originally speculated that the M form of K15 may have originated in a Neanderthal herpesvirus (Van Blerkom 2003), but the detection of the M form in Africa suggests that there would have been one or more unknown hominin populations who had contact with AMH in Africa and exchanged pathogen DNA with them. As speculated by Weiss (Weiss 2007), recent molecular evidence supports a hypothesis that humans acquired herpes simplex virus 2 (HSV-2) from chimpanzees 1.6 MYA through an intermediate hominid host (Severini et al. 2013; Wertheim et al. 2014). In a sense, these herpesvirus genomes are a fossil record, preserving evidence of past pathogenic interactions between hominids. Examples such as this inform our hypothesis that pathogen transfer between hominin populations took place in Eurasia during the Pleistocene.

If we consider candidate pathogens AMH may have transmitted to Neanderthals, *Helicobacter pylori* is a candidate: estimated to have first infected humans in Africa 88-116kya, carried out-of-Africa by AMH, and arriving in Europe after 52kya (Moodley et al. 2012). Chimpanzees do not harbour *H. pylori*, and there is evidence that some African hunter-gatherer groups, such as the Baka, did not acquire *H. pylori* until the last several hundred years, through contact with other groups (Nell et al. 2013). The same process of pathogen transmission may have occurred between Neanderthals and AMH.

The close genetic relatedness of AMH and other hominins would only have made it easier for pathogens to jump from one hominin population to another. In the Holocene, wild non-human primates have been the source of acute and chronic infectious diseases which have caused significant mortality: HIV, human T lymphotropic viruses (HTLVs), and vivax and falciparum malaria, for example (Liu et al. 2010; Liu et al. 2014; Trueba and Dunthorn 2012; Weiss 2001; Wolfe et al. 2007). This demonstrates the ability of infectious diseases to spread between species, through horizontal, vertical or vector-driven disease transmission routes. Humans migrating out of Africa would have been a significant reservoir of tropical diseases, not all of which require vectors for transmission. Likewise, the native Neanderthal populations of Eurasia would have carried hominin-adapted local microbes and parasites.

Conclusion

Analysing the genomes of archaic hominins and adaptively introgressed DNA carried by modern humans provides evidence of pathogens acting as a selection pressure (Prüfer et al. 2014). Through sequencing ancient pathogen DNA, excavating fossilised parasites (Anastasiou and Mitchell 2013; Mitchell 2013), and by utilising evidence that Neanderthals had genetic immunity to certain infectious diseases, we will be able to detect pathogens which were previously 'invisible' to paleopathology (Wood et al. 1992). Skeletal evidence is no longer the sole source of evidence of individual or group-level pathology. Studying genetic data (from host and pathogen) may also point towards new skeletal markers of infection. Comparison of skeletal remains from hominins and hunter-gatherers from the geographical range of the Neanderthals may identify infectious diseases which exerted a significant selection pressure on the Neanderthal genome, and provide evidence of selection on genetic pathways within the growing collection of ancient human, Neanderthal and Denisovan genomes.

Paleogenomic data must continue to inform our model of the first epidemiologic transition. The view of the Pleistocene infectious disease landscape is being enriched by analysis of modern and ancient human genomes. The period of Neanderthal adaptation and exposure to pathogens during the European Pleistocene was of much greater depth than AMH, and this long term exposure to local pathogens appears to have influenced the shape of both contemporary hominin genomes and their modern human descendants who still carry small

stretches of their DNA.

Omran (Omran 1971) considers parasitic diseases, tuberculosis, pneumonia (respiratory infection) and diarrhoeal diseases to be hallmarks of disease in the early agricultural era of the Holocene, dubbed “the age of pestilence and famine”. Anthropological and epidemiological data suggest that many acute infections require large, sedentary populations to be maintained, or an available pool of pastoral animals to act as intermediate hosts (Barrett et al. 1998), precluding the spread of many infectious diseases in the Pleistocene. In contrast, host and pathogen genetic data support a modified hypothesis of acute respiratory, soft tissue and diarrhoeal diseases having a pre-Holocene association with AMH (Armelagos and Harper 2005) and Neanderthals. Many of the pathogens thought to have originated in pastoral animals actually originated in humans, including, brucellosis, *Bordetella pertussis*, typhus, typhoid and perhaps tuberculosis. Subsequently, a number of these infections have passed to ruminants and poultry during the transition to agriculture and the intensification of farming (eg (Hoberg et al. 2001; Kidgell et al. 2002; Wirth et al. 2008)). Increased population densities, sedentism and the rise of agriculture during the Holocene may have intensified their impact on modern human health, changing disease transmission dynamics and increasing mortality rates. For the Neanderthal population of Eurasia, exposure to new human pathogens carried out of Africa may have been catastrophic.

The model of the first epidemiologic transition must continually develop to include new genetic data. We must also incorporate several hominin populations interbreeding and exchanging pathogens, not just AMH. The transition to the Holocene subsistence package may be most remarkable for changing disease dynamics rather than completely changing the Eurasian disease package. Further host and pathogen ancient DNA analysis will allow us to look afresh at relative impacts of migration, subsistence and interbreeding between hominin populations on the evolution of the modern human immune system and the infectious disease package in Eurasia.

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CJH and SJU declare no competing financial interests.

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