

1 **Neanderthal Genomics Suggests a Pleistocene Time Frame for the First Epidemiologic**
2 **Transition**

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12 **Abstract – 147 words**

13 High quality Altai Neanderthal and Denisovan genomes are revealing which regions of
14 archaic hominin DNA have persisted in the modern human genome. A number of these
15 regions are associated with response to infection and immunity, with a suggestion that
16 derived Neanderthal alleles found in modern Europeans and East Asians may be associated
17 with autoimmunity. Independent sources of DNA-based evidence allow a re-evaluation of
18 the nature and timing of the first epidemiologic transition. By combining skeletal,
19 archaeological and genetic evidence we question whether the first epidemiologic transition
20 in Eurasia was as tightly tied to the onset of the Holocene as has previously been assumed.
21 There clear evidence to suggest that this transition began before the appearance of
22 agriculture and occurred over a timescale of tens of thousands of years. The transfer of
23 pathogens between human species may also have played a role in the extinction of the
24 Neanderthals.

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26 **WORDS 3918**

27 **Introduction**

28 Current models of infectious disease in the Pleistocene tell us little about the pathogens
29 that would have infected Neanderthals (*Homo neanderthalensis*). If we consider the work of
30 Cockburn^{1,2}, Omran³, and Barrett⁴, who argue that infectious disease only started to
31 seriously impact human groups after the development of agriculture during the Holocene,
32 we must assume that Neanderthals lived in a time and place largely free of acute infectious
33 disease. The current epidemiologic transition model associates most infectious diseases that
34 cause significant mortality with changing living conditions connected with the rise of
35 agriculture, increased sedentism and higher population densities. As Pleistocene hunter-
36 gatherers, Neanderthals should not be at risk from the “pestilences” of Omran’s thesis.
37 However, new genetic evidence has the potential to change our view of Neanderthal
38 pathology, and perhaps even the model of the first epidemiologic transition.

39 Firstly, we must consider the current tools for studying infectious disease in the Pleistocene
40 period. Before the advent of ancient DNA sequencing methods, researchers were limited to
41 studying the skeletal pathologies of humans and Neanderthals from this period (fossilised
42 evidence of bones responding to infection and inflammation). However, only a limited
43 subset of infectious diseases leaves behind these lesions. The publication of high-quality
44 Neanderthal and Denisovan genomes gives us a new opportunity to study Pleistocene
45 infectious disease. Researchers from a range of disciplines interested in the evolution of the
46 modern suite of infectious disease can also draw inferences from this new source of data. As
47 a result of making comparisons between modern humans genomes, seeking genetic
48 polymorphisms which vary in function or frequency between populations, and by also
49 comparing human genomes with high-quality Denisovan and Neanderthal genomes, we are
50 beginning to find evidence of introgressed Neanderthal and Denisovan alleles and
51 haplotypes which have functions in immunity and the response to infection⁵⁻⁷. The
52 persistence of these regions of DNA in some modern human genomes suggests they may
53 have conveyed a selective advantage, increasing the fitness of anatomically modern humans
54 (AMH) when dispersing into new environments. Through comparisons of genetic data with
55 skeletal evidence of infection, it is increasingly to analyse which pathogens shaped the

56 evolution of modern humans and their closest relatives. Furthermore, ancient DNA
57 technology now encompasses pathogen DNA, and in the future it may be possible to
58 sequence pathogen DNA directly from Neanderthal remains – including pathogens that do
59 not cause skeletal lesions. The genomes of extant human pathogens are shedding light on
60 the antiquity of these infections in hominins.

61 We will discuss the evidence for infectious disease in Neanderthals, beginning with that of
62 infection-related skeletal pathologies in the archaeological record, and then consider the
63 role of infection in hominin evolution. We have synthesised current thinking on the
64 chronology of emergence of notable European disease packages (Table 1). Finally, we will
65 consider what implications this evidence has for the classical model of the first
66 epidemiologic transition.

67 **The Neanderthal Fossil Record**

68 *Homo neanderthalensis* was a large bodied hominin that inhabited Eurasia widely from
69 approximately 250,000 to 28,000 years ago⁸. Neanderthals occupied a hunter-gatherer
70 subsistence niche, forming small bands of approximately 15-30 individuals⁸. Archaeological
71 analysis suggests that while Neanderthal groups were relatively self-sufficient there was
72 some level of exchange and transfer of materials⁹ The Neanderthal fossil record of some 400
73 individuals represents one of the largest collections of extinct hominin remains and is larger
74 than that of contemporary Pleistocene *Homo sapiens* fossils. Genetic estimates for
75 Neanderthal population size vary but agree that total numbers were small. Most studies
76 suggest that the effective population size was between 3000-25000 (peaking around 50KYA
77 before gradual decline)¹⁰⁻¹⁴. Bocquet-Appel & Degioanni¹⁵ have suggested that the actual
78 population could have theoretically reached 70,000 but agree that the effective population
79 size would have been much smaller due to the impact of environmental and ecological
80 pressures. Thus, the fossil record presents researchers with a broad sample of the whole
81 population. Despite the breadth of skeletal material and the large number of pathological
82 lesions described, the Neanderthals are still viewed as existing in a hunter-gatherer
83 epidemiologic paradigm, an effect of the traditional approach of describing each fossil in
84 relative isolation. Conversely, while systematic population level studies have shown that the
85 Neanderthals sustained high levels of traumatic injury^{16,17}, the same methods have largely

86 not been applied to infectious disease. When reviewed as a species there is evidence that
87 along with traumatic injury the Neanderthals displayed a broad range of dental pathology
88 and degenerative diseases as well as a large amount of non-specific infection¹⁸⁻²¹.

89 From the perspective of the current epidemiologic transition model, the Neanderthals' small
90 group size and limited exchange networks suggests that they could not act as reservoirs for
91 infectious diseases. Also, much has been made of the Neanderthals' apparent lack of
92 cognitive and technological sophistication²². There is no logical reason to suppose infectious
93 diseases were unknown to Neanderthal groups based on the fossil evidence. Indeed, the
94 structure of Neanderthal groups would have made disease a potent factor in any
95 demographic collapse related to extinction events²³. As our understanding of Neanderthal
96 biology and behaviour becomes more sophisticated we are presented with a hominin which
97 was arguably every bit as intelligent and adaptive as *Homo sapiens*²⁴⁻²⁶. Their extinction and
98 our survival potentially questions any innate superiority in *Homo sapiens*. Recent genetic
99 analysis that suggests interbreeding further calls into question the real nature of the
100 divisions traditionally drawn between the two alpha human hominins.

101

102 **Innate, adaptive and archaic immunity in hominin genomes**

103 2010 saw the publication of the draft Neanderthal genome sequence²⁷, which revealed that
104 humans living outside Africa have a small proportion of Neanderthal ancestry – ~2% of their
105 genome²⁸. Three Neanderthal genome sequences are available: a draft sequence from
106 Vindija in Croatia, the composite sequence of DNA from bones from three different layers
107 (inferred to be from different individuals), dating from between 38-45kya²⁷; a low-coverage
108 sequence of a Neanderthal found in Mezmaiskaya in the Caucasus, from a layer dated as 60-
109 70kya; and a high-quality Neanderthal genome from the Altai region⁵, dated to 29-45kya.
110 The data set is growing constantly, recently bolstered by a 49kya Neanderthal exome
111 sequence (the ~1% of the genome which codes for proteins) from El Sidron in Spain, and a
112 further 44kya exome from Neanderthal remains recovered from Vindija²⁹. Comparisons of
113 these genome and exome sequences (although taken from only a handful of individuals) to
114 those of modern humans have identified several regions of genetic similarity between
115 humans and Neanderthals that are thought to have arisen from admixture between these

116 two hominins. Approaches to identifying introgressed Neanderthal regions in the human
117 genome which may be adaptive have looked for a range of different kinds of variation, from
118 haplotype blocks hundreds of kilobases long, to single nucleotide polymorphisms (SNPs).

119 One such putatively introgressed region plays a role in innate immunity to viral infections. A
120 haplotype containing *OAS1*, *OAS2*, *OAS3* of Neanderthal origin has been found in some
121 modern human genomes³⁰. These genes activate RNase L to degrade viral RNA. One
122 Neanderthal derived SNP in *OAS2*, rs15895, is associated with response to tick-borne
123 encephalitis virus disease in Europeans³¹. This is a disease found in forested areas of
124 northern, central and eastern Europe, which would have formed a major part the
125 Neanderthals' typical ecosystem^{8,32}. Did this pathogen represent a particular selection
126 pressure for AMH colonising Europe, unlike genetically adapted Neanderthals?

127 There is also evidence for Neanderthals contributing to the innate immune system in
128 modern Papua New Guinea. A study by Mendez³⁰ found a haplotype carrying three genes
129 (*STAT2*, *ERBB3*, *ESYT1*) to be absent in Africans, but present at variable frequencies outside
130 Africa, peaking at 54% in Melanesians. The divergence time for the putatively introgressed
131 Melanesian *STAT2* haplotype and the Neanderthal *STAT2* haplotype is 78kya, compared to a
132 divergence time between the Human Reference Sequence haplotype and Neanderthal
133 haplotype of 609kya. *STAT2* is involved in the interferon-alpha response to viral infections,
134 including dengue³³, influenza and measles³⁴. It is of note that *STAT2* interacts with other
135 putatively introgressed Neanderthal genes, discussed above: *OAS1-3*.

136 Sankararaman and colleagues⁷ scanned the genomes of modern Europeans and Asians for
137 evidence of individual SNPs that have introgressed from Neanderthals, a number of which
138 have been associated with immunity and auto-immunity in modern humans. One of the
139 most interesting results was a putative introgressed Neanderthal SNP in interleukin 18
140 (*IL18*), a gene with a central role in the innate immune response and the development of
141 bacterial sepsis. *IL18* expression is induced by products of both gram-positive and gram-
142 negative bacteria. There is evidence for antagonistic pleiotropy in the role of *IL18* in human
143 health and disease: IL18 induces interferon gamma, which can protect against infection; but
144 increased IL18 cytokine signalling is also associated with allergic reaction and development
145 of sepsis³⁵. The introgressed *IL18* SNP rs1834481 is associated with decreased serum IL18

146 levels. If Neanderthals were particularly at risk from bacterial sepsis, this could have created
147 a selection pressure for reduced *IL18* expression³⁶.

148 A Neanderthal allele was also identified in *TNPO3*⁷, a gene associated with increased risk of
149 systemic lupus erythematosus. There is some evidence to suggest that SLE may be triggered
150 by an aberrant response to infection³⁷. Further SNPs were identified which play a role in
151 Crohn's disease, both to increase and decrease susceptibility to this auto-immune disease. A
152 separate study of the same Altai genome by Vernot and Akey⁶ identified a Neanderthal
153 variant of *RNF34* in modern Asian and European genomes, a ring-finger protein with anti-
154 apoptotic functions that interacts with tumour necrosis factor.

155 There are regions of the genome in which Neanderthal DNA does not persist, seemingly
156 removed by purifying selection for disadvantageous phenotypes; the continued presence of
157 genetic variants associated with immunity in some European and Asian genomes suggests
158 that some Neanderthal haplotypes conferred a selective advantage to *Homo sapiens* during
159 the colonisation of Europe and East Asia. However, individual studies of Neanderthal-human
160 admixture use different methods to identify introgressed DNA, and subsequently identify
161 different regions of the human genome as Neanderthal-derived. It is unclear whether this
162 methodological diversity is a strength or a weakness of the field, as the false-positive rate is
163 unknown.

164 It is also important to note that our interpretation of the function of these genetic variants,
165 and our identification of immunity-related variants, relies upon our knowledge of the
166 function of genes and polymorphisms within the human genome, which is incomplete – for
167 example, there may be many more polymorphisms affecting susceptibility to viral, bacterial
168 or fungal infection which we have not yet identified in modern humans, and therefore
169 cannot identify in Neanderthal genetic data.

170 **Ancient pathogen genomics**

171 The work of Johannes Krause³⁸ and others^{39,40} raises the tantalising possibility of being able
172 to directly test ancient remains for evidence of infection by amplifying the DNA or RNA of
173 the pathogens which infected them in life. As the horizon for amplifying ancient host DNA
174 moves further back in time (most recently, the 400,000 year old mtDNA sequence from

175 Sima de los Huesos in Spain⁴¹), it is likely that amplifying ancient pathogen DNA from
176 selected skeletal remains of Neanderthals and Denisovans will become possible. Ancient
177 pathogen sequencing is even reaching into the mouths of Mesolithic and early Neolithic
178 individuals, characterising the oral pathogens preserved in dental calculus⁴². Environmental
179 contamination remains a significant issue in studies of ancient pathogen DNA, with careful
180 use of nucleic extraction methods and well-chosen controls necessary for its prevention and
181 identification⁴³.

182

183 **Infectious disease in the Pleistocene**

184 The paradigm of the first epidemiologic transmission, the hypothesis that epidemic disease
185 did not occur until the transition to agriculture, with larger, denser and more sedentary
186 populations, has been essentially unchallenged since the 1970s. Our views of the infectious
187 disease environment of the Pleistocene period are heavily influenced by skeletal data and
188 studies of contemporary hunter-gatherers¹. New genetic data – encompassing both hosts
189 and pathogens – has the power to transform our view of the infectious disease landscape
190 experienced by Neanderthals in Europe, and the AMH with whom they came into contact.
191 The Pleistocene hominin environment cannot be thought of as free from infectious disease.
192 It seems likely that the first epidemiologic transition, envisaged as part of the package of the
193 Holocene farming lifestyle, may be fundamentally different in pace or scope than has
194 previously been suggested.

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

196 **Challenging the Holocene epidemiologic transition**

197 In the genomes of Neanderthals we can clearly see evidence of the selection pressure
198 exerted by infectious disease. The genome of a 7,000-year-old hunter-gatherer from La
199 Brana in Spain shows similar signals of selection, for example carrying the non-functional
200 form of gene *CASP12* (caspase-12). Functional *CASP12* is associated with an increased risk of
201 bacterial sepsis, and the non-functional form is at or approaching fixation in non-African
202 populations⁴⁴. When considered alongside the reduced expression of Neanderthal *IL18* SNP
203 found in some Europeans and Asians, it is clear that bacterial sepsis was a significant

204 selection pressure on archaic and AMH, long before the assumed arrival of zoonoses with
205 the rise of agriculture in the Holocene.

206 Sequencing of the Neanderthal and Denisovan genomes has revealed a number of regions
207 of putatively introgressed archaic DNA in modern European and Asian genomes, as
208 discussed above, providing evidence that Neanderthals - and potentially other non-human
209 hominins - experienced significant selection pressure to adapt to infectious disease; these
210 same stretches of DNA may have been advantageous in protecting admixed AMH against
211 the same pathogens.

212 Paleogenomics provide us with a counterpoint to the AMH skeletal evidence of increasing
213 infectious disease in the Holocene, contributing to a view of Pleistocene Europe as riddled
214 with infectious diseases and parasites. Studying the phylogenetic relationships of extant
215 pathogens has led researchers to conclude that many infectious diseases have been co-
216 evolving with humans and our ancestors for tens of thousands to millions of years.
217 Furthermore, pathogens that were traditionally thought to be zoonoses acquired from herd
218 animals may in fact be anthroponoses, pathogens humans passed to their animals during
219 the rise of agriculture.

220 It is useful to consider which infectious diseases European Neanderthal populations may
221 have experienced (Table 1). Pleistocene diseases include pathogens which are found in all
222 primates, and are therefore likely to have co-speciated with Neanderthals (also known as
223 heirloom pathogens); and also those pathogens that phylogenetic evidence suggest predate
224 the Holocene, and are therefore potential Neanderthal pathogens. The same infectious
225 diseases would have affected the first AMH in Europe. They are compared to the diseases
226 associated with the transition to agriculture in the Holocene.

227 Certain pathogens are of particular interest to those studying infectious disease in
228 Neanderthals (see Table 1). Kuhn and colleagues⁴⁵ speculate that a Pleistocene European
229 rock shelter shows evidence of bedding being burned to eliminate parasites and pests. If
230 Pleistocene European AMH were subject to parasites contaminating their bedding,
231 Neanderthals must have been similarly burdened. There are significant tapeworm reservoirs
232 in African primates that have ancient divergence dates from other species⁴⁶ and both
233 Neanderthals and AMH were likely to have carried these parasites. The extent to which they

234 would have caused symptomatic disease is less clear: helminths are often thought to have
235 been a significant source of infectious disease for early foragers, but modern subsistence
236 farmers have higher helminth loads than modern foragers (with the caveat that modern
237 hunter-gatherers/foragers and farmers are not a time capsule of the Pleistocene or
238 Holocene disease landscape⁴⁷).

239 *Brucella* may be a very ancient human pathogen, despite its modern associations with milk
240 and pastoralism. Phylogenetic analysis of the *Brucella* genus suggests that the different
241 species of *Brucella* diverged tens of thousands of years before the origin of pastoralism and
242 has likely been endemic in wild animal populations for 80,000 – 300,000 years⁴⁸. Brucellosis
243 could therefore have been a disease of Neanderthals and AMH. There are skeletal reports of
244 brucellosis in *Australopithecus africanus*, an order of magnitude earlier than the above
245 estimates⁴⁹. Oral pathogens would also have been a hazard for Neanderthals, not just
246 Holocene farmers. There are reports of dental caries from *Homo heidelbergensis*⁵⁰.
247 Sequencing of Pleistocene dental calculus from AMH and Neanderthals would help
248 researchers to understand the evolution of oral microenvironments during the Pleistocene.

249 It is therefore likely that Neanderthals were subject to a wide variety of infectious diseases,
250 many of which do not leave skeletal lesions. These pathogens would have had the capacity
251 to cause morbidity and mortality in a variety of settings: infections of dental carries and
252 flesh wounds; childhood diseases (e.g. varicella zoster - chicken pox); gastrointestinal
253 infections; sexually transmitted infections; progressive infections such as leprosy; and many
254 chronic infections which would have been carried for life and only become symptomatic
255 when other infections led to immune suppression, such as tuberculosis and hepatitis.

256 **Disease exchange**

257 There is as yet no evidence of infectious disease transmission between AMH and
258 Neanderthals, but when considered in the light of the temporal and geographical overlap
259 between the two species⁵¹ and the evidence of admixture, it must have occurred. There is
260 compelling evidence from Africa of pathogen exchange between humans and other
261 hominins, preserved in the genome of Kaposi's sarcoma herpes virus (human herpesvirus 8).
262 The K15 gene of KSHV has three highly divergent forms, P, M and N. P is most common, M is
263 found at low frequencies worldwide, and N is rare and found solely in southern Africa⁵². It is

264 thought that the highly divergent M and N forms of K15 introgressed into human KSHV
265 strains through recombination with another herpesvirus that has yet to be detected in
266 modern humans. Based on the divergence dates of the different forms of K15, Hayward and
267 Zong suggest that the M form diverged from the P form 200,000 years ago, and the N form
268 500,000 years ago. The presence of these other K15 gene forms has arisen through contact
269 with other hominin species who carried their own KSHV-like viruses which speciated with
270 each hominin group. It was originally speculated that the M form of K15 may have
271 originated in a Neanderthal herpesvirus⁵³, but the detection of the M form in Africa - where
272 Neanderthal DNA is not detected in living humans - suggests that there would have been
273 one or more unknown hominin species who had contact with AMH in Africa and exchanged
274 pathogen DNA with them. In a sense, the KSHV genome is a fossil record, preserving
275 evidence of past pathogenic interactions between hominins.

276

277 *Helicobacter pylori* may be a pathogen which humans transmitted to Neanderthals. *H. pylori*
278 made the out-of-Africa migration with modern humans, estimated to have first infected
279 humans in Africa 88-116kya, and arriving in Europe after 52kya⁵⁴. Chimpanzees do not
280 harbour *H. pylori*, and there is evidence that some African hunter-gatherer groups, such as
281 the Baka, did not acquire *H. pylori* until the last several hundred years, through contact with
282 other groups⁵⁵. The same process of pathogen transmission may have occurred between
283 Neanderthals and AMH.

284

285 **Primates, hominins and zoonoses**

286 The close genetic relatedness of AMH and other hominins would only have made it easier
287 for pathogens to jump from one species to another. In the Holocene, wild non-human
288 primates have been the source of acute and chronic infectious diseases which have caused
289 significant mortality: HIV, human T lymphotropic viruses (HTLVs), and vivax and falciparum
290 malaria, for example⁵⁶⁻⁶⁰. This demonstrates the ability of infectious diseases to spread
291 between species, through horizontal, vertical or vector-driven disease transmission routes.
292 Humans migrating out of Africa would have been a significant reservoir of tropical diseases,

293 not all of which require vectors for transmission. Likewise, the native Neanderthal
294 populations of Eurasia would have carried hominin-adapted local microbes and parasites.

295 **Inbreeding depression and Neanderthal immunity**

296 The complete genome sequence of a Neanderthal from the Altai mountains (dated 50,000
297 B.P.) has also revealed a factor important to our understanding of infectious disease in
298 Neanderthals: inbreeding. The parents of the Altai Neanderthal were as closely related as
299 half-siblings or other similar relationships (e.g. double first cousins). This Neanderthal came
300 from a small effective population, where genetic heterozygosity was low⁵. Infectious
301 diseases would have become an increasingly important factor in Neanderthal mortality, as
302 genetic variants increasing susceptibility to infection became more common in the
303 population and the likelihood of infants being born with primary immune deficiencies
304 increased⁶¹.

305 **Conclusion**

306 Analysing the genomes of archaic hominins provides evidence of pathogens acting as a
307 population-level selection pressure, causing changes in genomes that were passed on to
308 descendants and preserved in the genomes of modern Eurasians. Through sequencing
309 ancient pathogen DNA, excavating fossilised parasites, and evidence that Neanderthals had
310 genetic immunity to certain infectious diseases, we will be able to detect pathogens which
311 were previously 'invisible' to paleopathology⁶². Skeletal evidence is no longer the sole
312 source of evidence of individual or group-level pathology. Studying genetic data (from host
313 and pathogen) may also point towards new skeletal markers of infection. Comparison of
314 skeletal remains from hominins and hunter-gatherers from the geographical range of the
315 Neanderthals may identify infectious diseases which exerted a significant selection pressure
316 on the Neanderthal genome, and provide evidence of selection on appropriate genetic
317 pathways within the growing collection of ancient human, Neanderthal and Denisovan
318 genomes.

319 Paleogenomic data must inform our model of the first epidemiologic transition. The view of
320 the Pleistocene infectious disease landscape is being radically altered by analysis of modern
321 and ancient human genomes. Pleistocene hominins were under considerable selection

322 pressure due to infectious disease and the fingerprints of this selection are preserved in
323 ancient and modern genomes. Selection pressure on Neanderthals and on AMH colonising
324 the Neanderthal range must have been maintained over a period of time, or the mutations
325 would have been lost from the modern human gene pool. Clearly, the Pleistocene in
326 temperate regions was not free from acute or chronic infectious diseases and the selection
327 pressures they exert.

328 Omran³ considers parasitic diseases, tuberculosis, pneumonia (respiratory infection) and
329 diarrhoeal diseases to be hallmarks of disease in the early agricultural era of the Holocene,
330 dubbed “the age of pestilence and famine”. Anthropological and epidemiological data
331 suggest that many acute infections require large, sedentary populations to be maintained,
332 or an available pool of pastoral animals to act as intermediate hosts⁴, precluding the spread
333 of many infectious diseases in the Pleistocene. In contrast, host and pathogen genetic data
334 suggest a hypothesis of acute respiratory, soft tissue and diarrhoeal diseases having a pre-
335 Holocene association with AMH and Neanderthals. Many of the pathogens thought to have
336 originated in pastoral animals actually originated in humans, including tuberculosis,
337 brucellosis, *Bordetella pertussis*, typhus and typhoid. Subsequently, a number of these
338 infections have become anthroponoses, infections that humans have passed to ruminants
339 and poultry during the transition to agriculture and the intensification of farming. Many of
340 the infectious diseases previously thought to be hallmarks of the first epidemiologic
341 transition (placed in the Holocene) have their roots in the Pleistocene. The rise of
342 agriculture during the Holocene may have intensified their impact on modern human health
343 and changed disease transmission dynamics, but it no longer makes sense to think of the
344 transition as a change from health to pestilence. Infectious disease was entrenched in the
345 Pleistocene landscape, and was an evident selection pressure for hominins in temperate and
346 tropical latitudes. For the Neanderthal population of Eurasia, exposure to new human
347 pathogens carried out of Africa may have been catastrophic.

348 The model of the first epidemiologic transition must continually develop to include new
349 genetic data. We must also consider whether the first epidemiologic transition was a much
350 longer process than previously envisaged, spanning both the Pleistocene and early Holocene
351 epochs and several hominin species, not just *Homo sapiens*. The analysis of ancient
352 genomes demonstrates that human behavioural patterns (in this case a shift to agricultural

353 subsistence) should not be used as an ecological proxy to explain shifting trends in the co-
354 evolutionary relationship between pathogens and human populations.

355 References

- 356 1. Cockburn, A. *The Evolution and Eradication of Infectious Diseases*. 255 (Johns Hopkins
357 Press, 1963).
- 358 2. Cockburn, T. A. Infectious Diseases in Ancient Populations. *Curr. Anthropol.* **12**, 45
359 (1971).
- 360 3. Omran, A. R. The epidemiologic transition: a theory of the epidemiology of
361 population change. 1971. *Milbank Q.* **83**, 731–57 (2005).
- 362 4. Barrett, R., Kuzawa, C. W., McDade, T. & Armelagos, G. J. EMERGING AND RE-
363 EMERGING INFECTIOUS DISEASES: The Third Epidemiologic Transition. *Annu. Rev.*
364 *Anthropol.* **27**, 247–271 (1998).
- 365 5. Prüfer, K. *et al.* The complete genome sequence of a Neanderthal from the Altai
366 Mountains. *Nature* **505**, 43–9 (2014).
- 367 6. Vernot, B. & Akey, J. M. Resurrecting surviving Neandertal lineages from modern
368 human genomes. *Science* **343**, 1017–21 (2014).
- 369 7. Sankararaman, S. *et al.* The genomic landscape of Neanderthal ancestry in present-
370 day humans. *Nature advance on*, (2014).
- 371 8. Davies, R. & Underdown, S. The Neanderthals: a Social Synthesis. *Cambridge*
372 *Archaeol. J.* **16**, 145 (2006).
- 373 9. HAYDEN, B. NEANDERTAL SOCIAL STRUCTURE? *Oxford J. Archaeol.* **31**, 1–26 (2012).
- 374 10. Bocquet-Appel, J.-P. & Demars, P.-Y. Population Kinetics in the Upper Palaeolithic in
375 Western Europe. *J. Archaeol. Sci.* **27**, 551–570 (2000).
- 376 11. Green, R. E. *et al.* Analysis of one million base pairs of Neanderthal DNA. *Nature* **444**,
377 330–6 (2006).
- 378 12. Green, R. E. *et al.* A complete Neandertal mitochondrial genome sequence
379 determined by high-throughput sequencing. *Cell* **134**, 416–26 (2008).
- 380 13. Fabre, V., Condemi, S. & Degioanni, A. Genetic evidence of geographical groups
381 among Neanderthals. *PLoS One* **4**, e5151 (2009).
- 382 14. Ghirotto, S., Tassi, F., Benazzo, A. & Barbujani, G. No evidence of Neandertal
383 admixture in the mitochondrial genomes of early European modern humans and
384 contemporary Europeans. *Am. J. Phys. Anthropol.* **146**, 242–52 (2011).
- 385 15. Bocquet-Appel, J.-P. & Degioanni, A. Neanderthal Demographic Estimates. *Curr.*
386 *Anthropol.* **54**, S202–S213 (2013).

- 387 16. Berger, T. D. & Trinkaus, E. Patterns of Trauma among the Neandertals. *J. Archaeol.*
388 *Sci.* **22**, 841–852 (1995).
- 389 17. Underdown, S. A comparative approach to understanding neanderthal trauma.
390 *Period. Biol.* **108**, 485–493 (2006).
- 391 18. Fennell, K. J. & Trinkaus, E. Bilateral Femoral and Tibial Periostitis in the La Ferrassie 1
392 Neanderthal. *J. Archaeol. Sci.* **24**, 985–995 (1997).
- 393 19. Antón, S. C. Endocranial hyperostosis in Sangiran 2, Gibraltar 1, and Shanidar 5. *Am. J.*
394 *Phys. Anthropol.* **102**, 111–22 (1997).
- 395 20. Ogilvie, M. D., Hilton, C. E. & Ogilvie, C. D. Lumbar anomalies in the Shanidar 3
396 Neanderthal. *J. Hum. Evol.* **35**, 597–610 (1998).
- 397 21. Dудay, H. & Arensburg, B. La pathologie. *Le squelette moustérien Kebara* 179–193
398 (1991).
- 399 22. Mellars, P. & French, J. C. Tenfold population increase in Western Europe at the
400 Neanderthal-to-modern human transition. *Science* **333**, 623–7 (2011).
- 401 23. Underdown, S. A potential role for transmissible spongiform encephalopathies in
402 Neanderthal extinction. *Med. Hypotheses* **71**, 4–7 (2008).
- 403 24. Soressi, M. *et al.* Neandertals made the first specialized bone tools in Europe. *Proc.*
404 *Natl. Acad. Sci. U. S. A.* **110**, 14186–90 (2013).
- 405 25. Lazuén, T. European Neanderthal stone hunting weapons reveal complex behaviour
406 long before the appearance of modern humans. *J. Archaeol. Sci.* **39**, 2304–2311
407 (2012).
- 408 26. Villa, P. & Roebroeks, W. Neanderthal demise: an archaeological analysis of the
409 modern human superiority complex. *PLoS One* **9**, e96424 (2014).
- 410 27. Green, R. E. *et al.* A draft sequence of the Neanderthal genome. *Science* **328**, 710–22
411 (2010).
- 412 28. Seguin-Orlando, A. *et al.* Genomic structure in Europeans dating back at least 36,200
413 years. *Science (80-.)*. science.aaa0114– (2014). doi:10.1126/science.aaa0114
- 414 29. Castellano, S. *et al.* Patterns of coding variation in the complete exomes of three
415 Neandertals. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 6666–71 (2014).
- 416 30. Mendez, F. L., Watkins, J. C. & Hammer, M. F. Neanderthal origin of genetic variation at
417 the cluster of OAS immunity genes. *Mol. Biol. Evol.* **30**, 798–801 (2013).

- 418 31. Barkhash, A. V *et al.* Variability in the 2'-5'-oligoadenylate synthetase gene cluster is
419 associated with human predisposition to tick-borne encephalitis virus-induced
420 disease. *J. Infect. Dis.* **202**, 1813–8 (2010).
- 421 32. Stewart, J. R. Neanderthals and modern humans. An ecological and evolutionary
422 perspective. C. Finlayson. Publisher Cambridge University Press, Cambridge, 2004
423 (265 pp.). ISBN: 0 521 82087 1. *J. Quat. Sci.* **21**, 206–207 (2006).
- 424 33. Mendez, F. L., Watkins, J. C. & Hammer, M. F. A haplotype at STAT2 Introgressed from
425 neanderthals and serves as a candidate of positive selection in Papua New Guinea.
426 *Am. J. Hum. Genet.* **91**, 265–74 (2012).
- 427 34. Chowdhury, F. Z. & Farrar, J. D. STAT2: A shape-shifting anti-viral super STAT. *JAK-*
428 *STAT* **2**, e23633 (2013).
- 429 35. Dinarello, C. A. & Fantuzzi, G. Interleukin-18 and host defense against infection. *J.*
430 *Infect. Dis.* **187 Suppl** , S370–84 (2003).
- 431 36. He, M. *et al.* Genome-wide association study identifies variants at the IL18-BCO2
432 locus associated with interleukin-18 levels. *Arterioscler. Thromb. Vasc. Biol.* **30**, 885–
433 90 (2010).
- 434 37. Cooper, G. S. *et al.* Hormonal, environmental, and infectious risk factors for
435 developing systemic lupus erythematosus. *Arthritis Rheum.* **41**, 1714–24 (1998).
- 436 38. Bos, K. I. *et al.* A draft genome of *Yersinia pestis* from victims of the Black Death.
437 *Nature* **478**, 506–10 (2011).
- 438 39. Biagini, P. *et al.* Variola virus in a 300-year-old Siberian mummy. *N. Engl. J. Med.* **367**,
439 2057–9 (2012).
- 440 40. Wagner, D. M. *et al.* *Yersinia pestis* and the plague of Justinian 541-543 AD: a
441 genomic analysis. *Lancet Infect. Dis.* **14**, 319–26 (2014).
- 442 41. Meyer, M. *et al.* A mitochondrial genome sequence of a hominin from Sima de los
443 Huesos. *Nature* **505**, 403–6 (2014).
- 444 42. Adler, C. J. *et al.* Sequencing ancient calcified dental plaque shows changes in oral
445 microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat. Genet.*
446 **45**, 450–5, 455e1 (2013).
- 447 43. Campana, M. G., Robles García, N., Rühli, F. J. & Tuross, N. False positives complicate
448 ancient pathogen identifications using high-throughput shotgun sequencing. *BMC*
449 *Res. Notes* **7**, 111 (2014).
- 450 44. Xue, Y. *et al.* Spread of an inactive form of caspase-12 in humans is due to recent
451 positive selection. *Am. J. Hum. Genet.* **78**, 659–70 (2006).

- 452 45. Kuhn, S. L. *et al.* The early Upper Paleolithic occupations at Uçağizli Cave (Hatay,
453 Turkey). *J. Hum. Evol.* **56**, 87–113 (2009).
- 454 46. Ravasi, D. F., O’Riain, M. J., Davids, F. & Illing, N. Phylogenetic evidence that two
455 distinct *Trichuris* genotypes infect both humans and non-human primates. *PLoS One*
456 **7**, e44187 (2012).
- 457 47. London, D. & Hruschka, D. Helminths and human ancestral immune ecology: What is
458 the evidence for high helminth loads among foragers? *Am. J. Hum. Biol.* **26**, 124–9
- 459 48. Foster, J. T. *et al.* Whole-genome-based phylogeny and divergence of the genus
460 *Brucella*. *J. Bacteriol.* **191**, 2864–70 (2009).
- 461 49. D’Anastasio, R., Staniscia, T., Milia, M. L., Manzoli, L. & Capasso, L. Origin, evolution
462 and paleoepidemiology of brucellosis. *Epidemiol. Infect.* **139**, 149–56 (2011).
- 463 50. Zanolli, C. & Mazurier, A. Endostructural characterization of the *H. heidelbergensis*
464 dental remains from the early Middle Pleistocene site of Tighenif, Algeria.
- 465 51. Higham, T. *et al.* The timing and spatiotemporal patterning of Neanderthal
466 disappearance. *Nature* **512**, 306–309 (2014).
- 467 52. Hayward, G. S. & Zong, J. C. Modern evolutionary history of the human KSHV genome.
468 *Curr. Top. Microbiol. Immunol.* **312**, 1–42 (2007).
- 469 53. Van Blerkom, L. M. Role of viruses in human evolution. *Am. J. Phys. Anthropol. Suppl*
470 **37**, 14–46 (2003).
- 471 54. Moodley, Y. *et al.* Age of the association between *Helicobacter pylori* and man. *PLoS*
472 *Pathog.* **8**, e1002693 (2012).
- 473 55. Nell, S. *et al.* Recent acquisition of *Helicobacter pylori* by Baka pygmies. *PLoS Genet.*
474 **9**, e1003775 (2013).
- 475 56. Wolfe, N. D., Dunavan, C. P. & Diamond, J. Origins of major human infectious
476 diseases. *Nature* **447**, 279–83 (2007).
- 477 57. Liu, W. *et al.* Origin of the human malaria parasite *Plasmodium falciparum* in gorillas.
478 *Nature* **467**, 420–5 (2010).
- 479 58. Liu, W. *et al.* African origin of the malaria parasite *Plasmodium vivax*. *Nat. Commun.*
480 **5**, 3346 (2014).
- 481 59. Trueba, G. & Dunthorn, M. Many neglected tropical diseases may have originated in
482 the Paleolithic or before: new insights from genetics. *PLoS Negl. Trop. Dis.* **6**, e1393
483 (2012).

- 484 60. Weiss, R. A. The Leeuwenhoek Lecture 2001. Animal origins of human infectious
485 disease. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **356**, 957–77 (2001).
- 486 61. Barbouche, M.-R. *et al.* Primary immunodeficiencies in highly consanguineous North
487 African populations. *Ann. N. Y. Acad. Sci.* **1238**, 42–52 (2011).
- 488 62. Wood, J., Milner, G. & Harpending, H. The osteological paradox: problems of inferring
489 prehistoric health from skeletal samples. *Curr. Anthropol.* (1992).
- 490 63. Hoen, A. G. *et al.* Phylogeography of *Borrelia burgdorferi* in the eastern United States
491 reflects multiple independent Lyme disease emergence events. *Proc. Natl. Acad. Sci.*
492 *U. S. A.* **106**, 15013–8 (2009).
- 493 64. Margos, G. *et al.* MLST of housekeeping genes captures geographic population
494 structure and suggests a European origin of *Borrelia burgdorferi*. *Proc. Natl. Acad. Sci.*
495 *U. S. A.* **105**, 8730–5 (2008).
- 496 65. Byun, R., Elbourne, L. D. H., Lan, R. & Reeves, P. R. Evolutionary Relationships of
497 Pathogenic Clones of *Vibrio cholerae* by Sequence Analysis of Four Housekeeping
498 Genes. *Infect. Immun.* **67**, 1116–1124 (1999).
- 499 66. Zink, A., Reischl, U., Wolf, H., Nerlich, A. G. & Miller, R. *Corynebacterium* in ancient
500 Egypt. *Med. Hist.* **45**, 267–272 (2001).
- 501 67. Levett, P. N. Leptospirosis. *Clin. Microbiol. Rev.* **14**, 296–326 (2001).
- 502 68. Comas, I. *et al.* Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium*
503 tuberculosis with modern humans. *Nat. Genet.* **45**, 1176–82 (2013).
- 504 69. Saunders, N. J., Hood, D. W. & Moxon, E. R. Bacterial evolution: *Curr. Biol.* **9**, R180–
505 R183 (1999).
- 506 70. Humphrey, L. T. *et al.* Earliest evidence for caries and exploitation of starchy plant
507 foods in Pleistocene hunter-gatherers from Morocco. *Proc. Natl. Acad. Sci. U. S. A.*
508 **111**, 954–9 (2014).
- 509 71. Diavatopoulos, D. A. *et al.* *Bordetella pertussis*, the causative agent of whooping
510 cough, evolved from a distinct, human-associated lineage of *B. bronchiseptica*. *PLoS*
511 *Pathog.* **1**, e45 (2005).
- 512 72. Kidgell, C. *et al.* *Salmonella typhi*, the causative agent of typhoid fever, is
513 approximately 50,000 years old. *Infect. Genet. Evol.* **2**, 39–45 (2002).
- 514 73. Farlow, J. *et al.* *Francisella tularensis* in the United States - Volume 11, Number 12—
515 December 2005 - Emerging Infectious Disease journal - CDC. *Emerging infectious*
516 *diseases* (2005). at <http://wwwnc.cdc.gov/eid/article/11/12/05-0728_article>

- 517 74. Woodford, M. H., Butynski, T. M. & Karesh, W. B. Habituating the great apes: the
518 disease risks. *Oryx* **36**, 153–160 (2002).
- 519 75. Lovell, N. C., Jurmain, R. & Kilgore, L. Skeletal evidence of probable treponemal
520 infection in free-ranging African apes. *Primates* **41**, 275–290 (2000).
- 521 76. Harper, K. N. *et al.* The sequence of the acidic repeat protein (arp) gene differentiates
522 venereal from nonvenereal *Treponema pallidum* subspecies, and the gene has
523 evolved under strong positive selection in the subspecies that causes syphilis. *FEMS*
524 *Immunol. Med. Microbiol.* **53**, 322–32 (2008).
- 525 77. Boutellis, A., Abi-Rached, L. & Raoult, D. The origin and distribution of human lice in
526 the world. *Infect. Genet. Evol.* **23**, 209–17 (2014).
- 527 78. Brooks, D. R. & Ferrao, A. L. The historical biogeography of co-evolution: emerging
528 infectious diseases are evolutionary accidents waiting to happen. *J. Biogeogr.* **32**,
529 1291–1299 (2005).
- 530 79. Carter, R. & Mendis, K. N. Evolutionary and Historical Aspects of the Burden of
531 Malaria. *Clin. Microbiol. Rev.* **15**, 564–594 (2002).
- 532 80. Hoberg, E. P., Alkire, N. L., de Queiroz, A. & Jones, A. Out of Africa: origins of the
533 *Taenia* tapeworms in humans. *Proc. Biol. Sci.* **268**, 781–7 (2001).
- 534 81. White, B. J., Collins, F. H. & Besansky, N. J. Evolution of *Anopheles gambiae* in
535 Relation to Humans and Malaria. (2011). at
536 <<http://www.annualreviews.org/doi/full/10.1146/annurev-ecolsys-102710-145028>>
- 537 82. Fares, M. A. & Holmes, E. C. A revised evolutionary history of hepatitis B virus (HBV).
538 *J. Mol. Evol.* **54**, 807–14 (2002).
- 539 83. Van Hemert, F. J. *et al.* Protein X of hepatitis B virus: origin and structure similarity
540 with the central domain of DNA glycosylase. *PLoS One* **6**, e23392 (2011).
- 541 84. Kapoor, A. *et al.* Characterization of a canine homolog of hepatitis C virus. *Proc. Natl.*
542 *Acad. Sci. U. S. A.* **108**, 11608–13 (2011).
- 543 85. Nakano, T. *et al.* New findings regarding the epidemic history and population
544 dynamics of Japan-indigenous genotype 3 hepatitis E virus inferred by molecular
545 evolution. *Liver Int.* **32**, 675–88 (2012).
- 546 86. Hemelaar, J. The origin and diversity of the HIV-1 pandemic. *Trends Mol. Med.* **18**,
547 182–92 (2012).
- 548 87. Sharp, P. M. & Hahn, B. H. The evolution of HIV-1 and the origin of AIDS. *Philos. Trans.*
549 *R. Soc. Lond. B. Biol. Sci.* **365**, 2487–94 (2010).

- 550 88. McGeoch, D. J., Rixon, F. J. & Davison, A. J. Topics in herpesvirus genomics and
551 evolution. *Virus Res.* **117**, 90–104 (2006).
- 552 89. Otani, M. *et al.* Phylogeography of Human T-lymphotropic Virus Type 1 (HTLV-1)
553 Lineages Endemic to Japan. *Trop. Med. Health* **40**, 117–24 (2012).
- 554 90. Bravo, I. G., de Sanjosé, S. & Gottschling, M. The clinical importance of understanding
555 the evolution of papillomaviruses. *Trends Microbiol.* **18**, 432–8 (2010).
- 556 91. Zhong, S. *et al.* Distribution patterns of BK polyomavirus (BKV) subtypes and
557 subgroups in American, European and Asian populations suggest co-migration of BKV
558 and the human race. *J. Gen. Virol.* **90**, 144–52 (2009).
- 559 92. Albariño, C. G. *et al.* High diversity and ancient common ancestry of lymphocytic
560 choriomeningitis virus. *Emerg. Infect. Dis.* **16**, 1093–100 (2010).
- 561 93. Delmas, O. *et al.* Genomic diversity and evolution of the lyssaviruses. *PLoS One* **3**,
562 e2057 (2008).
- 563 94. Pomeroy, L. W., Bjørnstad, O. N. & Holmes, E. C. The evolutionary and
564 epidemiological dynamics of the paramyxoviridae. *J. Mol. Evol.* **66**, 98–106 (2008).
- 565 95. Nyström, S. & Hammarström, P. Is the prevalent human prion protein 129M/V
566 mutation a living fossil from a Paleolithic panzootic superprion pandemic? *Prion* **8**, 2–
567 10 (2014).

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571 **Competing financial interests**

572 CJH and SJU declare no competing financial interests.

573 **Table 1: Comparison of European Pleistocene and Early Holocene Disease Packages.**

Pleistocene infectious disease	Ref	Holocene infectious disease	Ref
Bacteria			
Borrelia	63, 64	Cholera	65
Brucellosis	49, 48	Diphtheria	66
<i>Helicobacter pylori</i>	54	Leptospirosis	67
Mycobacterial infections (leprosy, TB)	68	<i>Neisseria gonorrhoea</i>	59,69
Oral pathogens (eg Streptococcus mutans)	70	Plague (<i>Yersinia pestis</i>)	38
Pertussis	71		
Salmonella typhi (typhoid)	72		
Staphylococci	2		
Tularaemia	73		
Yaws	74–76		
Parasites			
Head and body lice	60,77	Helminths	47
Pinworms	78	Plasmodium vivax	79
Tapeworm	80	Plasmodium falciparum	81
Whipworm	46		
Viruses			
Adenoviruses	53	Caliciviridae (eg norovirus)	53
Coronaviridae	53	Hepatitis B, C & E	82–85
Hepatitis A	53	HIV	86,87
Herpesviridae (eg chickenpox)	88	HTLV	89
Papillomaviridae (eg HPV)	90	Influenza	53
Polyomaviridae (eg BK virus)	91	Lymphocytic chorionic meningitis	92
Rhabdoviridae (eg rabies)	93	Measles	94

		Mumps	94
<i>Other pathogens</i>			
Prion disease	95		

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