Insights into hominin phenotypic and dietary evolution from ancient DNA sequence data

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A B S T R A C T
Nuclear genome sequence data from Neandertals, Denisovans, and archaic anatomically modern humans can be used to complement our understanding of hominin evolutionary biology and ecology through i) direct inference of archaic hominin phenotypes, ii) indirect inference of those phenotypes by identifying the effects of previously-introgressed alleles still present among modern humans, or iii) determining the evolutionary timing of relevant hominin-specific genetic changes. Here we review and reanalyze published Neandertal and Denisovan genome sequence data to illustrate an example of the third approach. Specifically, we infer the timing of five human gene presence/absence changes that may be related to particular hominin-specific dietary changes and discuss these results in the context of our broader reconstructions of hominin evolutionary ecology. We show that pseudogenizing (gene loss) mutations in the TAS2R62 and TAS2R64 bitter taste receptor genes and the MYH16 masticatory myosin gene occurred after the hominin-chimpanzee divergence but before the divergence of the human and Neandertal/Denisovan lineages. The absence of a functional MYH16 protein may explain our relatively reduced jaw muscles; this gene loss may have followed the adoption of cooking behavior. In contrast, salivary amylase gene (AMY1) duplications were not observed in the Neandertal and Denisovan genomes, suggesting a relatively recent origin for the AMY1 copy number gains that are observed in modern humans. Thus, if earlier hominins were consuming large quantities of starch-rich underground storage organs, as previously hypothesized, then they were likely doing so without the digestive benefits of increased salivary amylase production. Our most surprising result was the observation of a heterozygous mutation in the first codon of the TAS2R38 bitter taste receptor gene in the Neandertal individual, which likely would have resulted in a non-functional protein and inter-individual PTC (phenylthiocarbamide) taste sensitivity variation, as also observed in both chimpanzees and hominins.

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Ancient DNA-aided reconstruction of hominin evolutionary biology and behavior

Nuclear DNA sequence data from individuals of extinct hominin populations and species — and even archaic anatomically modern human populations — can be used to complement our paleoanthropological and archaeological understandings of hominin evolutionary biology and ecology. To date, high-coverage ancient DNA complete nuclear genome sequence data from two archaic hominin individuals have been published: i) a Neandertal (Homo neanderthalensis) from Denisova Cave in the Siberian Altai Mountains (~30x sequence coverage; Prüfer et al., 2014), and ii) a representative of a not-yet named but putatively distinct hominin population, herein referred to as a ‘Denisovan’ (~30x sequence coverage; Meyer et al., 2012), whose existence has been inferred through genomic sequence data recovered from a distal finger bone and an upper molar that were also collected at the same Denisova Cave site (Krause et al., 2010; Reich et al., 2010). Medium-to high-coverage DNA sequence data from nuclear gene coding regions only (the ‘exome’) are also now available from an additional two Neandertal individuals, one from El Sidrón Cave in Spain and one from Vindija Cave in Croatia (~12x and ~42x sequence coverage, respectively; Castellano et al., 2014).

Excitingly for paleoanthropologists, the discovery of relatively low levels of nuclear genome introgression from Neandertal and Denisovan populations to a subset of surviving modern human
populations (Reich et al., 2010; Meyer et al., 2012; Prüfer et al., 2014; Sankaraman et al., 2014; Vernot and Akey, 2014) provides not only an opportunity to study archaic admixture and population history, but also the ability to reconstruct major aspects of Neandertal and Denisovan biology in a relatively straightforward manner. That is, we can now study modern human phenotypic and genetic variation using genome wide association study (GWAS) approaches for any originally introgressed genome segment that remains in the modern human gene pool in order to infer the biological characteristics of Neandertals and Denisovans. While the value of this approach has been demonstrated on a case-by-case basis, for example with potential light skin-associated alleles in Neandertals (Vernot and Akey, 2014) and a high altitude-related adaptation in Denisovans (Huerta-Sanchez et al., 2014), we expect that anthropologists can look forward to the publication of much more comprehensive analyses along these lines in the coming years.

It is otherwise also possible, but considerably more difficult due to the need for functional or experimental validation, to make phenotypic inferences about Neandertals and Denisovans from the direct study of their nuclear genome sequences. The best current example is work on a Neandertal-specific melanocortin 1 receptor (MC1R) gene variant that likely conferred light skin and red hair phenotypes (Lalueza-Fox et al., 2007). A recent analysis identified a significant enrichment for Neandertal-specific nonsynonymous (amino acid-changing) substitutions within genes involved in skeletal development, specifically loricardic curvature (Castellano et al., 2014). The potential phenotypic consequences of these genetic changes have not yet been identified.

Finally, analyses of ancient DNA sequence data may benefit our reconstructions of hominin evolutionary biology and behavior by letting us infer the timing of relevant genetic changes that distinguish humans (Homo sapiens) from chimpanzees. Based on analyses of nuclear genome sequence data, Neandertals and Denisovans are more closely related to each other than either is to humans, with estimated Neandertal-Denisovan divergence of ~380 kya (thousands of years ago) and divergence of that lineage from the human lineage of ~550–590 kya (Prüfer et al., 2014). Medium-coverage ancient DNA nuclear genome sequence data have also been published for anatomically modern humans dated to ~12.6 kya from Montana, USA (~14x sequence coverage; Rasmussen et al., 2014), ~4 kya from Greenland (~20x sequence coverage; Rasmussen et al., 2010), ~7 kya and ~8 kya from Europe (~19x and ~22x sequence coverage, respectively; Lazaridis et al., 2014), with other nuclear genomes from similarly-dated or even older modern human individuals published at lower coverage (e.g., Keller et al., 2012; Lazaridis et al., 2014; Olalde et al., 2014; Raghavan et al., 2014). Thus, this particular approach has limited resolution — we are presently only able to determine whether the hominin-specific gene changes in question occurred prior to or more recently than ~600 kya, or between this time and the relatively recent past. Yet, such analyses still have the potential to provide insight into the timing of hominin phenotypic evolution and behavioral ecology transitions.

Hominin dietary evolution

In this article, we illustrate the method and potential value of the evolutionary timing approach through consideration of published ancient DNA nuclear genome sequence data for genes and genetic changes with hypothesized relevance to outstanding questions of hominin dietary evolution. A number of major dietary transitions have occurred during the ~6 million years of hominin evolution, including substantial increases in the consumption of meat and starch, the cooking of food, and the domestication of plants and animals (Wrangham and Conklin-Brittain, 2003; Ungar et al., 2006; Luca et al., 2010). These transitions, in turn, have hypothesized to have played critical roles in other major aspects of hominin evolution, including encephalization, molar size reduction, dispersals out of Africa, pair bonded social structure, and sedentism (e.g., Leonard and Robertson, 1992; Aiello and Wheeler, 1995; Milton, 1999; Stanford, 1999; Wrangham et al., 1999; Mann, 2000; Diamond, 2002; Carmody and Wrangham, 2009; Wrangham and Carmody, 2010). Therefore, understanding the nature and timing of these major dietary transitions is important for our broader reconstruction of hominin evolutionary ecology. However, with the exception of the agricultural transition, given the rarity or absence of direct evidence from the hominin fossil record there is considerable uncertainty — or even strong debate — about the time periods during which these dietary shifts occurred (de Heinzelin et al., 1999; Laden and Wrangham, 2005; Bunn, 2006; McPherron et al., 2010; Roebroeks and Villa, 2011; Gouwlett and Wrangham, 2013).

The relationship between DNA sequence and phenotype is relatively simple for some diet-related genes. This is due, at least in part, to the specific functional roles of digestive enzymes and taste receptors. Multiple diet-related gene presence/absence substitutions distinguish modern humans from chimpanzees; functional interpretations are made more readily for these molecular differences than for individual nucleotide substitutions at non-synonymous (amino acid changing) or regulatory sites. The timings of associated phenotypic changes in our evolutionary history that may inform our understanding of hominin dietary transitions are thus readily interpretable through the analysis of ancient DNA sequence data. Here we review or determine the evolutionary timing of all non-olfactory receptor, hominin-specific diet-related gene function gain and loss changes that are known to us, discuss the potential significance of these results, and look forward to potential future extensions of this approach.

Diet-related gene losses in hominin evolution

First consider a gene whose functionality has been conserved and maintained by purifying selection (i.e., functional constraint) across most or all studied mammals, over hundreds of millions or billions of years of combined evolutionary history, but then lost sometime during hominin evolution. Based on our knowledge of the single nucleotide and small insertion/deletion mutation rates we can infer that potential gene inactivating mutations — those that i) introduce a premature stop codon, ii) obliterate a critical exon/intron splice site, iii) alter the start codon, or iv) result in a coding sequence frameshift and thereby alter all downstream amino acids — occur regularly (e.g., Yamaguchi-Kabata et al., 2008; Yngvadottir et al., 2009; MacArthur and Tyler-Smith, 2010; MacArthur et al., 2012). Typically, functional gene loss via one of these mutational mechanisms would be detrimental to individual fitness and therefore the mutation would be removed from the population by purifying natural selection. However, changes in an organism’s environment or behavior (or, possibly, compensatory evolution at other genes) may obviate the function of the gene’s encoded protein product or make it less critical to individual fitness, such that pseudogenizing mutations may then increase in frequency and become fixed in a population or species.

A good example of this phenomenon is the convergent functional loss of the blue opsin gene (OPN1SW) in many nocturnal mammals, including some nocturnal primates, resulting in monochromatic vision (Jacobs et al., 1986; Tan et al., 2005; Bowmaker and Hunt, 2006). Under this framework, if we have knowledge about the function of a gene in question and can make inferences about the environmental or behavioral changes that likely
preceded or were associated with its functional loss, then studies of the timing of those gene losses can help to reconstruct the evolutionary and ecological history of a species in ways that complement and extend information available through the fossil record.

We analyzed the Neandertal and Denisovan high-coverage complete nuclear genome sequence data (Supplementary Online Material [SOM]) to study the evolutionary history of four potential diet-related genes harboring hominin lineage-specific pseudogenizing substitutions or function-eliminating mutations: MYH16, TAS2R62, TAS2R64, and TAS2R38. As an anatomically modern human ancient DNA control, we assessed the medium-coverage nuclear genome sequence data from the ~12.6 kya Montana individual using identical methods. The sarcomeric myosin gene MYH16 is expressed specifically in the masticatory muscles of non-human primates, but the full protein cannot be produced in humans due to an invariant 2 bp (base pair) deletion in exon 18 that results in a frameshift of the downstream amino acid sequence and a subsequent premature stop codon (Stedman et al., 2004). Presumably as a result, temporalis muscle Type II fibers are significantly smaller in humans than in macaques, and the loss of this gene may at least partly explain the greatly reduced masticatory muscle apparatus in humans relative to other catarhine pri-

mates (Stedman et al., 2004). We hypothesize that the functional loss of a gene that otherwise encodes an important masticatory muscle protein may very well have followed hominin control of fire and the advent of consistent cooking behavior, which results in substantial food softening and reduces demand on the masticatory apparatus (Wrangham and Conklin-Brittain, 2003; Dominy et al., 2008).

TAS2R62, TAS2R64, and TAS2R38 are bitter taste receptors. In TAS2R62 and TAS2R64 there are two and one premature stop codon mutations, respectively, that are observed among all humans and are hominin-specific among studied primates, although orangutans do have a different premature stop codon mutation that likely also inactivates their TAS2R64 gene (Parry et al., 2004; Go et al., 2005; Wang et al., 2006). To the best of our knowledge, the specific bitter molecules targeted by the functional versions of these receptors are not yet known. Such a functional assessment would be of potential value for the reconstruction of hominin dietary evolution, because those TAS2R proteins possibly target substances that are common in the diets of most or all great ape species but were absent from or less prevalent in the hominin environment or dietary intake over at least part of our evolutionary history.

In contrast, the hominin-specific functional mutations in the TAS2R38 gene are not fixed among humans, and they also do not result in a true pseudogene. Rather, there is a common haplotype at this gene containing nonsynonymous mutations corresponding to amino acid positions 49, 262, and 296. The protein encoded by the ancestral haplotype responds to the bitter compound phenylthio-

carbamide (PTC), whereas that encoded by the derived haplotype responds to the bitter compound phenylthio-

amino acid positions 49, 262, and 296. The protein encoded by this gene containing nonsynonymous mutations corresponding to this gene containing nonsynonymous mutations corresponding to this gene containing nonsynonymous mutations corresponding to

likely also inactivates their

This possibility could be formally excluded once nuclear genome sequences become available from Neandertal or Denisovan population samples.

In the absence of information on the compounds recognized by the proteins encoded by the TAS2R62 and TAS2R64 bitter taste receptors, we are currently unable to make specific inferences about hominin dietary history or evolutionary ecology on the basis of this information. However, the functional MYH16 protein is expressed in masticatory muscles; its absence in humans may explain our relatively reduced jaw muscles and Type II muscle fiber size (Stedman et al., 2004). We suggest that the hominin-specific loss of this protein may have followed the adoption of cooking behavior, which would have reduced constraints on the masticatory apparatus. Reductions in molar and gut size are commensurate with a marked increase in brain size beginning ~1.9 mya (millions of years ago; Aiello and Wheeler, 1995; Organ et al., 2011). This time period has been inferred as the potential origin of hominin cooking behavior (Wrangham et al., 1999; Wrangham and Carmody, 2010), despite the absence of direct evidence from the fossil record.

Previously, Stedman et al. (2004) studied the rates of human-specific nonsynonymous (amino acid changing) and synonymous MYH16 substitutions to algebraically estimate a ~2.4 mya date for the MYH16 frameshift deletion. Specifically, their dating method assumed steady functional constraint on nonsynonymous mutations prior to pseudogenization followed by an equal rate of non-

synonymous and synonymous substitution after gene loss. However, their analysis was based on human-chimpanzee differences at only one codon, surveyed from only a very short segment of MYH16 (Perry et al., 2005). Thus, additional approaches, such as the one applied in this study, are necessary to obtain more confidence in the time period in which this pseudogenizing mutation occurred. Our finding that the Neandertal and Denisovan individuals shared with humans the MYH16 frameshift deletion is consistent with both Stedman et al.’s (2004) ~2.4 mya estimate for the loss of this gene and the ~1.9 mya inference for the origins of hominin cooking behavior. The addition of nuclear genome sequence data from other archaic hominin species (see below) would provide a more precise estimate of the timing of the MYH16 gene loss, which in turn would have implications for different hypotheses concerning the origins of hominin cooking behavior.

The Neandertal individual is heterozygous for a novel TAS2R38 start codon-disrupting mutation.

In humans, three common amino acid polymorphisms in the TAS2R38 bitter taste receptor gene explain the majority of human

MYH16, TAS2R62, and TAS2R64 pseudogenizing mutations occurred prior to ~600 kya

We aligned the Neandertal and Denisovan sequence reads to the hominin-specific pseudogenes MYH16, TAS2R62, and TAS2R64. The pseudogene-causing substitutions are a 2 bp frameshift deletion in the sarcomeric myosin gene MYH16, and two and one stop codon mutations in the TAS2R62 and TAS2R64 bitter taste receptor genes, respectively. Both the Neandertal and Denisovan consensus sequences were identical to the human reference sequence in all cases (Fig. 1), with no evidence of any variation within either individual at these sites. Thus, the functionality of these three genes was likely lost prior to the ~550–590 kya divergence of the human and Neandertal/Denisovan lineages. We note the remote possibility, which we consider very unlikely given the age and location of the sequenced archaic hominins, that the derived Neandertal and Denisovan variants at these genes reflect admixture with anatomically modern humans, rather than the shared ancestry of pseudogenizing mutations that occurred earlier in hominin evolution. This possibility could be formally excluded once nuclear genome sequences become available from Neandertal or Denisovan population samples.
Surprisingly, we found that the Siberian Altai Neandertal individual was heterozygous for a mutation in the second nucleotide of the first codon of the TAS2R38 gene, similar to the common variant observed in chimpanzees, but here a T-C rather than a T-G polymorphism. Of the 19 aligned sequence reads at this position, 11 had the sequence T, and 8 = C (Fig. 2), providing strong evidence of a true polymorphism rather than a sequencing error. This single nucleotide polymorphism (SNP) would typically affect a Methionine to Threonine substitution, but again, since this is the start codon we can predict that this mutation results in a truncated and non-functional protein, similar to the chimpanzee variant (Wooding et al., 2006). Thus, human, chimpanzee, and Neandertal populations may all have had variable PTC taste sensitivity, in each case due to different functional mutations.

In the absence of nuclear genome sequence data from better population samples of archaic hominins, we cannot determine whether this Neandertal allele occurred at intermediate frequency, similar to the chimpanzee mutation and the human TAS2R38 functional polymorphisms. We also cannot yet exclude the possibility that similar variation existed in the Denisovan population. Still, this very interesting result raises at least two hypotheses for further testing, including with population genomic analyses: i) balancing selection for TAS2R38 functional polymorphism is common for hominins and non-human apes — although specific adaptive hypotheses are largely lacking (Wooding, 2006), and ii) the TAS2R38 gene is subject to only very weak purifying selection, such that mutations with major functional effects have attained intermediate frequency but not become fixed in any of these lineages. Either finding could help advance our understanding of hominin dietary evolution, especially if we could comprehensively catalog the natural food items targeted by this receptor.
Diet-related gene functional gains in hominin evolution

On the other side of the gene content spectrum, duplications or regulatory sequence changes affecting diet-related genes, especially digestive enzymes, may provide a selective advantage under certain environments or behaviors if they lead to functional increases in gene and protein expression. However, the evolutionary reconstructions and interpretations that can be made based on the timing of gene duplications and individual sequence changes are less direct than those from gene losses. Specifically, whole gene duplications or very specific regulatory mutations must first occur (randomly) before selection can potentially act to maintain them. For single-copy DNA sequence these mutational mechanisms are much less commonplace than the expectedly steady rate of pseudogenization mutations (as described above). Thus, we cannot make strong ecological conclusions from the absence of gene duplication or functionally relevant regulatory mutation at a given point in evolutionary history. However, the evolutionary origins of gene duplications and known regulatory sequence changes are still useful to consider because (depending on the duplication) these results may still help us make inferences about the fitness potential of a species under hypothesized environments or behaviors.

The origins of lactase persistence mutations are consistent with the agricultural transition

Lactase, encoded by the LCT gene, is the enzyme responsible for the digestion of the milk sugar lactose. Expression of the LCT gene in the small intestine is typically abolished sometime after weaning in mammals, including for the majority of modern human individuals. However, modern humans in multiple agriculturalist and pastoralist populations have continued expression of LCT, or 'lactase persistence,' throughout adulthood (Ingram et al., 2009). At least three independent mutations in a regulatory region located upstream of the LCT gene are responsible for the developmental gain in enzyme expression in different populations (Bersaglieri et al., 2004; Tishkoff et al., 2007; Heyer et al., 2011; Gallego Romero et al., 2012; Peng et al., 2012). These mutations are associated with genomic backgrounds that suggest past histories of strong positive selection occurring within the past 10 kya or less (Bersaglieri et al., 2004; Tishkoff et al., 2007; Ranciaro et al., 2014), consistent with the origins of agriculture.

Since the specific mutations that confer the lactase persistence phenotype in modern human populations are known, it has been possible to study the presence or absence of these mutations in ancient DNA nuclear genome sequence data from European anatomically modern humans in order to more precisely estimate the origins and spread of this phenotype (Itan et al., 2009). These studies have revealed an absence or relatively low frequency of the common European LCT persistence allele across ~10–5 kya and even more recent time periods (Burger et al., 2007; Nagy et al., 2011; Plantinga et al., 2012; Sverrisdottir et al., 2014), but then a frequency of ~70% by AD 1200 in at least one population in Germany (Kruttli et al., 2014). Thus, nuclear ancient DNA data have confirmed a likely relatively recent and agricultural/pastoral-associated origin for at least one of the mutations conferring human lactase persistence.

AMY1 duplications are human-specific and likely occurred within the past ~600 kya

Amylase is the enzyme responsible for the initial stages of the digestion of starch, which is a major dietary component for modern human agricultural populations (up to 70% of caloric intake;
Fig. 3. Salivary amylase (AMY1) gene copy number analysis. (A) Plots of read depth across the AMY1 region. Light shading indicates regions analyzed to estimate AMY1 copy number, via comparison of the AMY1 duplicated region (‘region for CNV estimate’) to the non-duplicated, non-deleted control region (‘diploid control region’). Dashed lines indicate the median read depth across the diploid control region, and y-axes scale to 5x the median. Sequence read coverage for each individual was capped at 1.5x the median of the duplicated AMY1 segment in order to remove repetitive element artifacts. For the plots only (not for the copy number estimation), the coverage curves were smoothed using a rolling median in a window equal to 1% of the contig length, then a 500 nt rolling window mean. Every 100th base position is represented on the curve. (B) Barplot of AMY1 copy number estimates derived by comparing AMY1 duplicated segment and diploid control region read depths. (C) Comparison of AMY1 read depth copy number estimates to array-based comparative genomic hybridization (aCGH) relative intensity log2 ratios for AMY1-mapped clone Chr1tp-6D2 for the modern human samples (Redon et al., 2006).
similar observation was also noted in the supplementary information of the Prüfer et al. (2014) Neandertal genome paper.

To validate our AMY1 copy number sequence read depth method, we compared our modern human results to those from a previous aCGH study conducted on the same individuals. Specifically, we compared our diploid copy number estimates to the relative intensity log₂ ratios for the Chr1tp-6D2 clone that is mapped to the AMY1 locus from Redon et al. (2006). The ratios reflect the relative fluorescence intensities from a competitive hybridization experiment in which dye-labeled DNA from each individual is separately co-hybridized to a microarray with the dye-labeled DNA (using a different dye) of a common reference sample. In regions of the genome in which an individual has a higher copy number than the reference sample (and vice versa for lower copy number), relatively more DNA from that individual will hybridize to the clones on the array containing DNA complementary to that region, resulting in a measurable skew in the intensities of the two fluorescent dyes. Thus, the Chr1tp-6D2 log₂ ratios reflect the relative AMY1 copy number among the 15 samples. These values are strongly and significantly correlated with our sequence read depth-based estimates of AMY1 diploid copy number in the same individuals (Fig. 3C; Pearson correlation test; \( r^2 = 0.84; \ P = 1.73 \times 10^{-5} \)), confirming the reliability of this approach.

Finally, we performed two analyses to test the sensitivity of this method to detect duplications in the archaic hominins. First, we selected four additional genomic regions containing both a segment with known duplication in the human genome and a non-duplicated, non-deleted control region. In each case, clear relative copy number gains were observed in the Neandertal and Denisovan individuals, similar to the modern human samples (SOM Figure 1). Second, we used identical methods to estimate 3.54 diploid AMY1 copies from the medium coverage nuclear genome sequence dataset of the anatomically modern human individual from Montana (Fig. 3). Thus, the absence of Neandertal and Denisovan AMY1 relative copy number gain signals reflects the absence of AMY1 duplications in the archaic hominins, rather than any lack of power to detect duplications with ancient DNA sequence data and this method. We can therefore conclude that AMY1 gene duplications are likely human-specific and that they occurred following the divergence of our lineage from the Neandertal/Denisovan lineage ~550–590 kya.

The three copies of AMY1 that are annotated in the human reference genome sequence are very similar to each other, which would seem to imply an origin within the past ~200 kya (Perry et al., 2007). However, tandem duplication sequence divergence may not scale with age, due to ongoing duplication and deletion and potential gene conversion mechanisms among the copies. Thus, an independent assessment of the likely origin of these duplications was necessary. Our ancient DNA results — only two diploid AMY1 copies in both the Neandertal and Denisovan individuals — are consistent with a relatively recent date for the AMY1 duplications (but one prior to the origins of agriculture, as evidenced by the presence of duplications in all anatomically modern human hunter-gatherers thus far studied with ancient DNA). This timing is not consistent with the hypothesized importance of starch-rich underground storage organs beginning in much earlier time periods of hominin evolution (Coursey, 1973; Wrangham et al., 1999; Laden and Wrangham, 2005). However, as noted in the Introduction, we cannot make strong inferences from the absence of gene duplications at a particular time in hominin evolutionary history. We can only conclude that if early hominins were consuming large quantities of starchy foods, as hypothesized, then they were likely doing so without the digestive benefits of increased salivary amylase production (Mandel and Breslin, 2012).

Concluding remarks

Looking forward, there are several ways in which the ancient DNA evolutionary timing of diet-related genetic changes approach could be expanded in order to better complement the limited information from the fossil record concerning hominin dietary transitions. Similar discussion points would also generally apply to investigations of other, non-diet-related hominin phenotypes (Sams et al., 2014). First, nuclear DNA sequence data from representatives of additional hominin lineages would facilitate more precise dating of hominin-specific DNA sequence changes. One interesting candidate is Homo floresiensis, which survived until ~18 kya in Indonesia, where the relatively hot and wet climate is not conducive to DNA preservation. However, with continuing advances in sequencing technology and ancient DNA methods such as single-strand DNA library preparation (Meyer et al., 2012) and ultra-short read sequencing (Dabney et al., 2013; Meyer et al., 2014), success may be possible in the future.

Second, our inferences will become stronger with better functional knowledge of specific taste receptors, including those that have been lost along the hominin lineage. Likewise, we would like to generally extend our analysis to specific nonsynonymous and gene regulatory substitutions of many potential diet-related genes in the genome, instead of focusing more heavily on hominin-specific gene gains and losses. However, functional knowledge of such changes is severely lacking for fixed human-chimpanzee differences, due to the difficulty in predicting phenotype from genotype in the absence of within-species variation.

Finally, the timing of olfactory receptor gene gains and losses and functional changes could provide insights into hominin dietary evolution and evolutionary ecology. This analysis is currently challenged by our limited understanding of the specific functional consequences of olfactory receptor variation and by the short sequence reads typically obtained from ancient DNA, which often cannot be mapped uniquely to specific, highly similar olfactory receptor genes (Hughes et al., 2014). We are hopeful for progress on all of these fronts.

In summary, we have demonstrated that analyses of high-coverage ancient DNA sequence data can facilitate inferences about the relative timing of hominin-specific phenotypic changes in diet-related genes. This analysis reveals the utility of ancient DNA approaches for addressing questions about the timing of evolutionary changes correlated with ecological shifts in hominin history, including those that are strongly debated by paleoanthropologists due to the limitations of the direct fossil record. As both our functional knowledge of genetic changes and the number of nuclear genome sequences from ancient hominin individuals expand, our ability to test such hypotheses and provide novel insights about hominin evolution will continue to improve.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jhevol.2014.10.018.
Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., 
Bartram, F., Renne, P., WoldeGabriel, G., Beyene, Y., 
Berger, J., Kirchner, M., Bramanti, B., Haak, W., Thomas, M.G., 2007. Absence of the
Biagi, R., Kharit, R., Wadie, M., Stade, B., Ake, M., Mayer, J., Spangler, J.,


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